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Structural anatomy of the breast

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Declaration

I, *Kathryn Maree Gaskin*, declare that this thesis is submitted in partial fulfilment of the requirements for the conferral of the degree *Masters of Philosophy*, from the University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. This document has not been submitted for qualifications at any other academic institution.

Kathryn Maree Gaskin

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Abstract

Introduction: The female breast, as originally described by Cooper in 1840[1], is a fundamental part of the reproductive system, and therefore is included in essentially every anatomy textbook [2-32]. Surprisingly, there has been very little anatomical research conducted on the breast since Cooper's work[1]. Cooper's [1] descriptions of the anatomy of the breast were extensive. His methodology however lacked detail in terms of the number of cadavers that anatomical descriptions were based on or any quantitative data to support his descriptions.

Despite this, most of the current anatomical descriptions of the breast within both textbooks and the literature are based on Cooper's [1]. Only 17 dissection studies have been published since Cooper [1] on the gross anatomy of the breast [33-48], with a paucity of published research found on the internal fascial structure of the breast. Although these studies have provided some additional anatomical detail of the breast, they have also lacked quantitative data. *In vivo* studies using magnetic resonance imaging (MRI) and ultrasound, and surgical studies investigating mastectomy tissue have provided some data and further anatomical detail of breast composition and structure [49-56]. To date however, the published descriptions of the gross anatomy of the breast have not been to be collated to form a consistent anatomical description of the internal gross anatomy for anatomical education. Therefore the aim of this study was to provide quantitative data on the gross anatomy of the breast to provide evidence-based detail for anatomical illustrations and descriptions on the gross anatomy of the breast.

Methods: A cadaveric-based investigation was carried out on embalmed female cadavers. The investigation focused on three (3) aspects of the female breast; ***(i)*** B***reast composition, (ii) Gross anatomical structure, and the (iii) Attachments of the breast to***

the chest wall. Eighteen breasts from nine cadavers were investigated quantitatively and the gross anatomy was described qualitatively using this data. A range of dissection techniques were used to investigate the breast in two planes, coronal and sagittal to provide a three dimensional understanding of the structure of the breast.

The coronal dissections were conducted from a superficial to deep dissection (n=15) with quantitative measurements taken at each depth of dissection. The sagittal dissections were conducted on slices of different breasts (n=18 slices, n=3 breasts). Different quantitative measurements were recorded in the sagittal plane compared to the coronal plane.

Results: ***Composition***

The mean total breast surface area and volume amongst the 15 breasts measured in the coronal plane was 302±91 cm^2 (range: 187-501 cm^2) and 381±272 mL (range: 56-959 mL). The mean total fibro-adipose and fibro-glandular mass per breast of the 14 breasts dissected in the coronal plane was 172±103 g (range: 50–385 g) and 184±77 g (range: 56-300 g) respectively. Breast composition by mass in terms of the percentage of fibro-glandular and fibro-adipose tissue was approximately 48% fibro-adipose tissue (range: 45-54 %). A moderate correlation was found between total breast mass and breast volume (r^2=0.577).

Gross Anatomical Structure

The mean number of fibro-adipose tissue pockets per coronal breast was 199±53 (range: 108 – 306). The number of pockets per breast was found to increase both with breast volume (r^2=0.737) and surface area (r^2=0.806; $P<0.05$). The mean size per pocket was (mass) 0.4±0.15 g (range: 0.26-0.61 g) and (surface area) 0.88±0.37 cm^2 (range: 0.31-1.97 cm^2). The fibro-adipose pockets found anterior to the gland were larger in size (mean: 0.89±0.32 cm^2, range: 0.34-1.20 cm^2) but less in number (mean: 35±16, range:

20-49) compared to those posterior to the gland (mean size: 0.33±0.22 cm^2, range: 0.22-0.87 cm^2; mean number: 53±31, range: 36-78). The mean length of the Anterior Extensions of the Anterior Lamellae was 19±5 mm (range: 9-34 mm).

Attachment of the breast to the chest wall

The perimeter attachment of the breasts was consistently found to be stronger than the Posterior Extensions of the Posterior Lamellae. The inferior perimeter was considered to be the strongest attachment due periosteal attachments and required sharp dissection.

The sternal head of Pectoralis Major muscle was found within the perimeter of all of the 12 breasts and made up the largest mean surface area (81%), followed by the Serratus 10%), External Oblique (5%) and Rectus Abdominus muscles (4%).

Conclusion: This study adds to the understanding of the structural anatomy of the breast by providing evidence to base new and more accurate illustrations of the gross anatomical structure of the breast and written descriptions of the ***composition***, ***gross anatomical structure*** and the ***attachments of the breast to the chest wall***. It can be used to update the structural anatomy of the breast in anatomical texts used to teach medical, allied health and science undergraduate students.

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Chapter 1:

Introduction

The female breast is a fundamental part of the reproductive system, and therefore is included in essentially every anatomy textbook recommended for undergraduate and graduate students in medicine and allied health courses within Australian Universities [2-32] (Table 1 and Table 2). Surprisingly, there has been very little anatomical research conducted on this region of the body, with the most extensive research conducted by Cooper in 1840[1]. Cooper's [1] publication of his breast dissections were extensive and included descriptions of the structure, composition and attachments of the breast to the chest wall. However, research at that time typically did not provide detailed methodology in terms of the number of cadavers that anatomical descriptions were based on or any quantitative data of the anatomical structures dissected. Despite this, most of the current anatomical descriptions of the breast within both textbooks and the literature are based on Cooper's [1] descriptions, and hence also lack any quantitative data to support or verify them. Only 17 dissection studies have been published since Cooper [1] on the gross anatomy of the breast [33-48] (Table 3), with a paucity of published research found on the internal fascial structure of the breast.

Although these studies have provided some additional anatomical detail of the breast, they have also lacked quantitative data to support their descriptions and many textbooks present conflicting descriptions of the internal fascial structure of the breast.

In-vivo studies using magnetic resonance imaging (MRI) and ultrasound, and surgical studies investigating breast tissue removed during breast mastectomy have provided further anatomical detail of breast composition and structure [49-56]. They have also provided some quantitative data on breast anatomy, composition and structure. To date however, the published descriptions of the gross anatomy of the breast have not been collated to form a consistent anatomical description of the internal gross anatomy for anatomical education.

The aim of this literature review was therefore to collate and summarise all of the relevant descriptions and research on the gross anatomy of the breast. This was achieved by two methods (i) a literature review of the published research, including the work of Cooper, (ii) a textbooks review. The literature review was conducted using the Scopus database for English articles from 1800-2016, using keywords female breast AND anatomy OR fibrous tissue OR glandular tissue OR adipose tissue OR Cooper's Ligaments OR suspensory ligaments OR adipose tissue AND NOT cancer AND NOT chicken. The terms cancer and chicken were included in an attempt to eliminate results relating to breast cancer or chicken breasts. Studies were included if they met the following selection criteria: i) examined breast anatomy using anatomical dissection techniques; ii) included female breasts, though no criteria was imposed in regards to the minimum number of breasts; iii) data presented in English. A total of 17 dissection studies were included in the literature review [33-48, 57]. A limitation of this study however is the exclusion of non-English works which may have re-examined and expanded on the breast anatomy originally investigated by Cooper [1].

Anatomical textbooks used in major universities around Australia for undergraduate or post-graduate medicine and allied health degrees were included in the textbook review of breast anatomy (Table 1). All of the major universities were contacted by phone by the Chief Researcher and their websites reviewed. Thirty-one textbooks were identified and included in the analysis (Table 2) [2-32]. The majority of the anatomical textbooks directed at introductory level anatomy for medical and allied health students (Table 1) only contained a small amount of written text regarding the gross anatomy of the breast, with one illustration of the breast. Considerable variation was found in the gross anatomy presented in these illustrations, and the majority of illustrations were of a relatively small, pert shaped breast, with very limited associated written text providing further detail of the illustration. Most of the written text within the anatomical textbooks focused on the regional anatomy or the fibro-glandular tissues of the breast. Not one textbook presented evidence or quantitative data to support either their illustrations or written text (Table 2).

As Cooper's [1] descriptions were so extensive and form the basis of current anatomical descriptions of the breast, the anatomical descriptions of the gross anatomy of the breast presented in the literature and textbooks were summarised and compared to the Cooper's [1] descriptions. The anatomy of the breast was investigated in the following sections: (i) composition; (iii) gross anatomical structure; (iv) attachments of breast to chest wall.

Table 1: Prescribed and recommended textbooks for undergraduate and graduate students in medicine and allied health at the major universities in Australia. (compiled as of June 2017 from personal conversations and online subject descriptions)

University	Textbook: Author (Year)
Australian National University - ACT	Drake et. al. (2005), Marieb and Hoehn (2012), McKinley and O'Loughlin (2006), Moore, Agur et. al. (2015)[7, 17, 18, 21]
Canberra University- ACT	Tortora et. al. (2015)[31]
Griffith University- Queensland	Marieb and Hoehn (2012)[17]
James Cook University - Queensland	Drake et. al. (2005), Marieb and Hoehn (2012), McKinley and O'Loughlin (2006), Moore, Agur et. al. (2015)[7, 17, 18, 21]
La Trobe University- Victoria	Drake et. al. (2005), Moore, Dalley et. al. (2014)[7, 22]
Macquarie University - New South Wales	Tortora and Derrickson (2012)[30]
Melbourne University - Victoria	Drake et. al. (2005), Eizenberg et. al. (2008), McMinn (1991), Moore, Dalley et. al. (2014)[7, 8, 19, 22]
Queensland University of Technology - Queensland	Clemente (2007), Olson (1996)[6, 24]
RMIT- Victoria	Abrahams et. al. (2008), Drake et. al. (2005), Moore, Agur et. al. (2015), Moore, Dalley et. al. (2014), Tortora and Derrickson (2012), Rohen, Yokochi et. al. (2006)[3, 7, 21, 22, 30, 58]
Southern Cross University- Queensland	Marieb and Hoehn (2012)Marieb and Hoehn (2012)
Sydney University – New South Wales	Agur and Dalley (1991), Drake et. al. (2005), Kapit and Elson (1993), Moore, Dalley et. al. (2014), Rohen et. al. (2006)[4, 7, 15, 22, 58]
University of Adelaide - South Australia	Agur and Dalley (1991), Drake et. al. (2005), Kapit and Elson (1993), Moore, Dalley et. al. (2014), Rohen et. al. (2006)[4, 7, 15, 22, 58]
University of New England - New South Wales	Tortora et. al. (2015)[31]
University of Newcastle - New South Wales	Gilroy et. al. (2012)[11]
University of Queensland - Queensland	Drake et. al. (2005), Hansen (2014), Rohen et. al. (2006)[7, 13, 58]
University of Tasmania - Tasmania	Drake et. al. (2005), Gilroy (2013), Hansen (2014), Moore, Dalley et. al. (2014), Rohen et. al. (2006), Martini et. al. (2008)[7, 10, 13, 22, 58, 59]
University of Technology Sydney - New South Wales	Marieb and Hoehn (2012)[17]
University of Western Australia - Western Australia	Saladin (2007)[26]
University of Wollongong – New South Wales	Marieb and Hoehn (2012) [17]
UNSW- New South Wales	Marieb and Hoehn (2012), Tortora and Derrickson (2012), Tortora et. al. (2015) [17, 30, 31]
Western Sydney University - New South Wales	Tortora et. al. (2015)[31]

Table 2: Summary (n=31) of undergraduate and post-graduate introductory level anatomy textbooks for students undertaking medial, health and science degrees in Australia.

Author (Year)	Sagittal Image (n)	Coronal Image (n)	Fibro-adipose structure	Fibro-glandular structure	Composition	Attachment to chest wall	Based on Data
Abrahams, Cravem et. al. (2005)[2]	✓	✓	✓	✓	✓	✓	
Abrahams, Boom et. al (2008)[3]	✓	✓					
Agur & Dalley (1991)[4]	✓	✓	✓	✓		✓	
Applegate (2000)[5]	✓	✓	✓	✓		✓	
Clemente (2007)[6]	✓	✓	✓	✓		✓	
Drake et. al. (2005)[7]		✓	✓	✓	✓	✓	
Eizenberg et. al. (2008)[8]					✓		
Ellis (2002)[9]		✓		✓		✓	
Gilroy (2013)[10]	✓	✓	✓	✓	✓	✓	
Gilroy et. al (2012)[11]	✓	✓	✓			✓	
Gosling et. al. (2003)[12]	✓			✓		✓	
Hansen (2014)[13]	✓	✓	✓	✓		✓	
Hansen & Lambert (2008)[14]	✓	✓		✓		✓	
Kapit & Elson (1993)[15]	✓	✓	✓	✓	✓		
Lindsay (1995)[16]	✓	✓	✓	✓			
Marieb & Hoehn (2012)[17]	✓			✓			

✓=included in the textbook

Table 2: ctnd.

Author (Year)	Sagittal Image (n)	Coronal Image (n)	Fibro-adipose structure	Fibro-glandular structure	Composition	Attachment to chest wall	Based on Data
McKinley & O'Loughlin (2006)[18]		✓					
McMinn (1991)[19]			✓	✓		✓	
Moffat (1993)[20]		✓		✓		✓	
Moore, Agur et. al. (2015)[21]	✓	✓	✓	✓		✓	
Moore, Dalley et. al (2014)[22]	✓	✓	✓	✓		✓	
Moses et. al. (2005)[23]	✓	✓		✓		✓	
Olson (1996)[24]		✓					
Rohen (2016)[25]	✓	✓					
Saladin (2007)[26]	✓	✓	✓	✓	✓	✓	
Sinnatamby (2006)[27]			✓	✓		✓	
Snell (1995)[28]	✓	✓	✓	✓		✓	
Tank & Gest (2008)[29]	✓	✓					
Tortora & Derrickson (2012)[30]	✓	✓	✓	✓		✓	
Tortora et. Al (2015)[31]	✓	✓	✓	✓		✓	
Van De Graff et. al. (2002)[32]	✓	✓	✓	✓		✓	
Total number of textbooks	23 (31 images)	26 (58 images)	19	24	6	22	0

✓=included in the textbook

Table 3: Cadaveric dissection studies (n=17) since Cooper to examine the breast and the research areas they contributed to.

Author (Year)	Sagittal Image (n)	Coronal Image (n)	Fibro-adipose structure	Fibro-glandular structure	Composition	Attachment to chest wall	Other	Based on Data
Arnez et. al. (1995) [33]		✓					✓ Internal mammary veins and intercostal spaces	✓
Bayati and Seckle (1995)[34]	✓	✓				✓		
Boutros et. al. (1997)[35]	✓					✓		
Cardoso et. al. (2015)[36]		✓	✓			✓		✓
Jinde et. al. (2006)[37]		✓	✓			✓	✓ Pectoral fascial thickness	✓
Klock et. al. (2016)[38]		✓	✓	✓			✓Validating QT* ultrasound	✓
Komiya et. al. (2015)[39]		✓	✓				✓ Nipple areolar complex	✓
Lockwood (1991)[40]	✓	✓	✓			✓	✓	
Matousek et. al. (2014)[41]	✓	✓	✓			✓		
Muntan et. al. (2000)[42]			✓			✓		
Nanigian et. al. (2007)[43]		✓				✓		✓
Parks (1959)[44]				✓				
Riggio et. al. (2000)[45]	✓	✓	✓			✓		
Sarhadi et. al. (1996)[46]	✓	✓					✓ Nerve supply	
Suami et. al. (2008)[47]		✓					✓ Lymphatics	
Wűringer et. al. (1998)[48]	✓	✓				✓	✓ Horizontal septum	
Total number of studies	6 (9 images)	13 (50 images)	8	2	0	10	8	6

✓=investigated this aspect of breast anatomy, *QT is a type of ultrasound

1.1 Composition

1.1.1 Literature Review

Cooper [1] described the breast to consist of four types of tissues; the overlying skin, glandular tissue, fibrous tissue and adipose tissue. He did not however quantify the relative percentage composition of each of these tissues or the volume, surface area or mass of the breast. There has been a paucity of anatomical research on the structural composition of the breast, consequently, anatomical descriptions and quantitative data on breast composition is also lacking in anatomical textbooks. Although Cooper's [1] anatomical illustrations provided some information on the composition of the breast, his illustrations were not labelled or associated with a figure caption. Therefore limited detail of relative tissue composition can be taken from them. Since Cooper's work[1], no published dissection study was found that specifically aimed to dissect and quantify the anatomical composition of the breast.

Breast volume, mass and surface area [60, 61] data has been collected in technology-based *in vivo* studies using three-dimensional scanning, MRI and mammography, casting and with anthropometric, measurements, and via surgical studies of mastectomy specimens using water displacement and tissue weighing [49-54, 61-63]. The breast volumes measured have ranged from 75 to 3100 mL [49-54, 61-63] with the mean breast volume varying from 273 [62] to 715 mL [49]. The variation in the mean volume measured can be attributed to the large range of breast volumes that exist in the female population and the differences in the volume range of the cohorts recruited within each study. Breast volume has also been found to vary with BMI [61] and age [49].

The variation in the mean volume measured amongst different studies can also be attributed to the different measurement techniques utilised to measured breast volume [50, 64] and inter-rater variation [62]. Studies that have utilised different breast volume measurement techniques such as scanning, water displacement, and MRI on the same cohort of breasts have found variation in the magnitude of volume measured [50, 62]. Surgical studies that have measured breast volume from mastectomy specimens [51] may also contain corrupted tissue, i.e. cancerous tissue. Only one study was found that attempted to quantify the regional distribution of breast volume [65]. The breasts of 176 women were scanned using three-dimensional scanning and the resultant scan was divided into quadrants through the nipple. The study reported the upper-inner quadrant to have the greatest distribution of breast volume and that the volume of the quadrants was positively and strongly correlated with the total breast volume (r^2=0.81–0.91, n=176)

Only one published study was found that measured breast mass [51]. A study of 21 complete mastectomy specimens following pure multifocal in situ carcinomas found breast mass to range from 110 to 1100 g, with a mean of 420±261 g. However, similar to the breast volume data from mastectomy studies, the breast tissue may also contain cancerous tissue, affecting the accuracy of this measurement.

Although *in vivo* breast volume and mass data exists, it has not been translated into anatomical texts. This may be related to the wide range of breast volumes measured and the variation in mean breast volume measured in different cohorts of women measured with different measurement technique. Further research is therefore required to quantify breast volume and breast mass based on quantitative data obtained from dissection specimens to update anatomical illustrations and descriptions presented in anatomical textbooks.

Due to the limited detail of the anatomical structures within the breast visualised by MRI and mammography compared to anatomical dissection research, breast composition research based on such technologies has been broadly quantified as a percentage of fibro-glandular and adipose tissue. Although a mean of 66±18 % adipose tissue was found in younger women (400 women, mean age 20.8 years[49]) and 70±22 % adipose tissue in older women (100 women, mean age 49.6 years[49]), the relative percentage of fibro-glandular and adipose tissue has also been found to vary with age, race, mass, hormonal changes and pregnancy [49, 51, 55, 66]. It has also been found to vary with measurement method. A study comparing breast composition determined from MRI and mammography on the same cohort of women (40 women, range: 20-83 years [55]) reported the mean percentage of adipose tissue within the breast to be 66.5±18 % when measured with MRI, and 42.5±30.3 % when measured on the same breast with mammography[55]. The authors attributed the differences to changes to the shape of the breast during the two different measurement methods. The breast deforms in the different body positions sustained during measurement, prone for MRI and standing for mammography.

Breast composition measured with mammography has also varied in different cohorts of women. Lee et. al. (1997)[55] reported the mean percentage of adipose tissue to be 42.5±30.3 % in 40 women between the ages of 20-83 years, while Engelken et. al. (2014)[52] reported the mean percentage of adipose tissue to account for 79% of tissue in the breast in 174 women between the ages of 20-79 years (mean age: 55 years) [52]. Unfortunately both studies included a very large age range in their cohort of relatively small numbers. Therefore the breast composition data attained from technology based *in vivo* studies is limited due to the lower level of visualisation of anatomical tissue in comparison to anatomical dissection studies and due to the discrepancies in the composition measured using different measurement technologies.

Although surgical studies measuring breast composition allow for greater visualisation of the tissues of the breast they many involve pathological breast tissue removed during mastectomy surgery [51]. In a surgical study of 21 complete mastectomy specimens (age range: 27-83; following pure in situ carcinomas)[51] breast composition was determined by measuring the adipose tissue volume using water-displacement. The mean percentage of adipose tissue volume was reported to be 24.5±12.8 %, with a range 7-56 % of total breast volume[51]. A limitation of this study however, was that the breasts also contained pathological tissue. Consequently, the data may not accurately represent the composition of the whole breast. Surgical studies are therefore limited in comparison to dissection studies and may not accurately represent the tissue composition and structure of the whole female breast [67].

Two literature review papers described the composition of the breast in different regions of the breast [68, 69]. Both papers divided the breast into quadrants and reported the superior-lateral quadrant to have a higher composition percentage of fibro-glandular tissue. However, these descriptions were not based on quantitative data and were not related to a particular cohort.

No anatomical dissection study to date has quantified breast volume, breast surface area, breast mass or breast composition. Although technology based in-vivo studies and surgical studies investigating breast composition have provided some quantitative data of fibro-glandular and adipose tissue composition, these studies are limited in their ability to accurately detail the breast composition compared to dissection studies. There is also considerable variability in breast composition with different measurement methods. Therefore further research is required to quantify the composition of the cadaveric breast mass to inform anatomical descriptions and illustrations.

1.1.2 Textbook review

Despite the lack of quantitative data on breast composition in the literature, anatomical textbooks have provided some detail of breast composition through anatomical illustrations more than through written text. Across the 31 textbooks included in the summary, there were 58 coronal illustrations of the breast (from 26 textbooks, Table 2) and 31 sagittal illustrations (from 23 textbooks, Table 2, Figure 1). Only three textbooks [8, 19, 27] had no illustrations within their breast anatomy section. The coronal images generally depicted the composition of the partially dissected breast to include the skin, fascial tissue, glandular tissue and adipose tissue with the Tail of Spence projecting into the axilla, consistent with Cooper [1]. Of the 26 textbooks that contained coronal images, three textbooks had cadaveric images [3, 23, 25] and four textbooks had surface anatomy photographs [2, 3, 6, 18]. Of the 26 textbooks that contained coronal images, 15 depicted the tissues that make up the breast (Table 2), however the relative quantitative composition of these tissues and their relative location was either inconsistent or not clearly quantified from the illustrations. Furthermore, no quantitative data or evidence was provided to verify the breast compositions presented.

Of the 23 textbooks with sagittal images (Table 2, Figure 1), five textbooks had cadaveric photographs [3, 12, 23, 25, 26] and one textbook had surface anatomy photographs [2]. Of the 31 sagittal images within the 23 textbooks, 24 were pure sagittal plane sections through the nipple [4, 6, 10-16, 23, 24, 29-32] and seven were from an anterior-medial aspect [2, 5, 17, 21, 22, 26, 28]. Twenty of the sagittal illustrations provided some detail of the composition of the breast [3-6, 10-17, 21, 22, 25, 26, 29-32] however it was not specifically quantified. Although the sagittal images were consistent in their location of the glandular tissue within the breast, they were inconsistent in terms of both the quantitative composition of the glandular, fibrous and adipose tissues within the breast and the relative locations of the fibrous and adipose tissues within the breast. For example, eight textbooks displayed adipose tissue both anterior and posterior to the glandular tissue [5, 13, 14, 26, 29-32], while five displayed adipose tissue only anterior to the glandular tissue [10, 11, 16, 17] and four displayed no discernible difference between the tissues of the breast [4, 6, 21, 22]. Only three textbooks contained cadaveric images to display breast composition and all of these were poorly labelled or captioned [3, 12, 25]. None of the textbooks included any quantitative data or referenced any studies to verify the breast composition basis of their illustrations.

The written text of the majority of the anatomical textbooks did not include any detail of the relative composition of the tissues within the breast. Indeed, breast composition was only mentioned in six textbooks [2, 7, 8, 10, 15, 26], which only described the types of tissue within the breast (adipose, fibrous and glandular tissues), not their relative composition. One textbook stated that breast mass was related to the increase in the adipose tissue mass (Table 2), however no data or evidence was provided to support this notion.

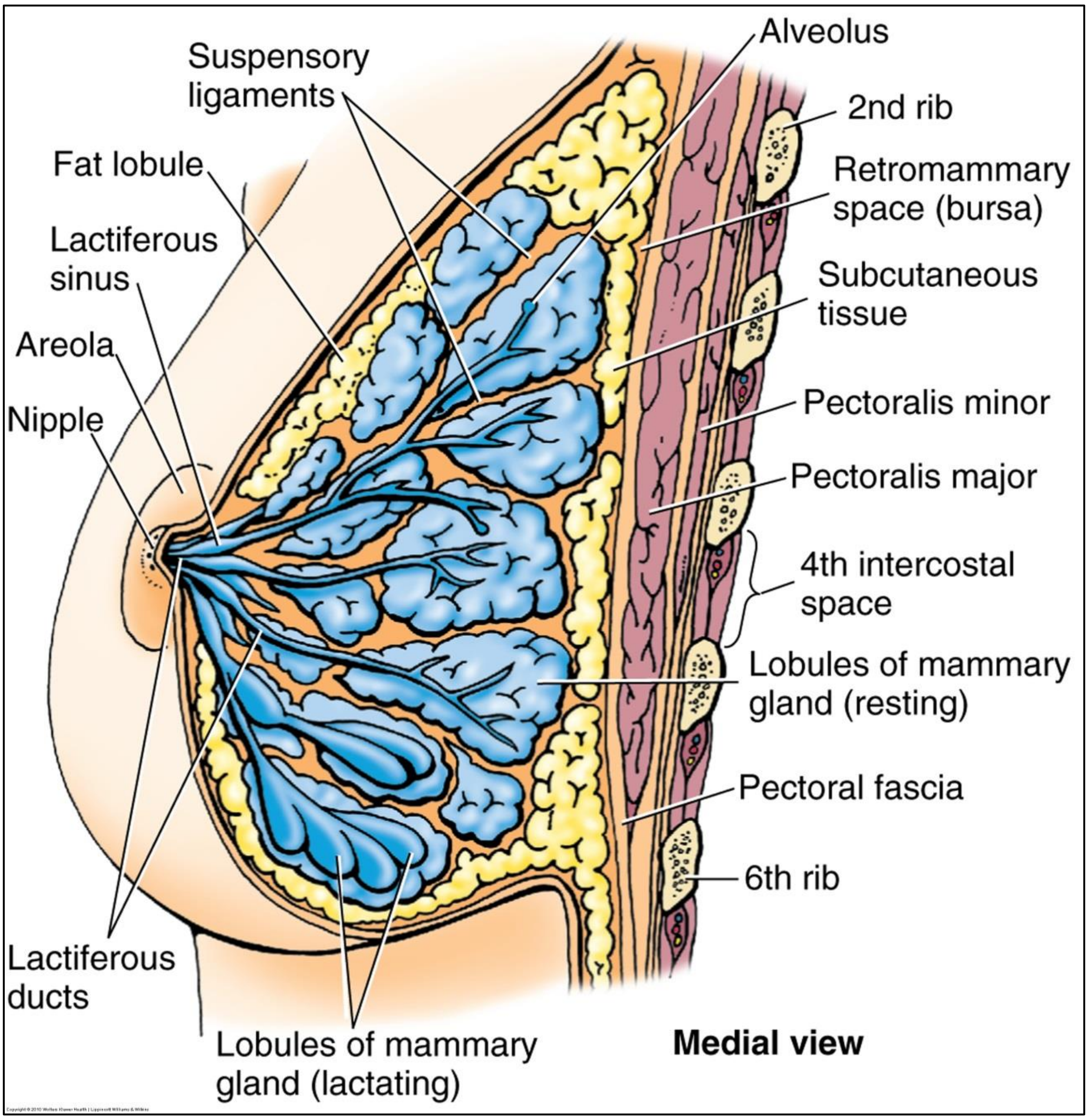

Figure 1 Example of textbook representation of the composition of the breast (Moore, Agur et. al. (2015)[21])

1.2 Gross Anatomical Structure

1.2.1 Fibro-Adipose Structure (Literature Review)

Anterior and Posterior Lamellae

The fibro-adipose structure of the breast was extensively described and illustrated (Figure 2) by Cooper [1] as a compartmentalised structure, consisting of encased lobules of fat which was located between two distinct fascial sheets, which he called the Anterior and Posterior Lamellae. Cooper [1] stated the Anterior Lamellae was adhered to the anterior surface of the mammary gland and the Posterior Lamellae was adhered to the posterior surface of the mammary gland. The two fibrous tissue layers of the breast (Anterior and Posterior Lamellae) developed from the mesenchyme which split embryonically at the inferior aspect of the sternum to encase the mammary gland. Anterior and Posterior extensions of each lamella were described to project anteriorly and posteriorly from each lamella (Anterior and Posterior Extensions) [1].

Although Cooper's[1] descriptions of the fibro-adipose structure of the breast were extensive, they were limited to the region covered by the gland and its associated Anterior Lamella and Posterior Lamella. The glandular tissue however was not described by Cooper [1] to cover the entire surface area of the breast, and therefore did the Anterior Lamella and Posterior Lamella. The anatomy fibro-adipose complex beyond the boarder of the glandular tissue however was consequently not described by Cooper[1]. His written text describing the fibro-adipose structure and his illustrations of it, were unfortunately located in different sections of his work, and his illustrations had no specific figure captions or labels. Consequently, Cooper's [1] description of the fibro-adipose structure of the breasts is limited as it lacks quantitative data, is incomplete in its structure and open to interpretation due to a lack of connection between his illustrations and his written text.

Since Cooper, only eight cadaveric dissection studies have investigated the fibro-adipose tissue of the breast [36-42, 45]. Many of these studies visualised, in consensus with Cooper, a dual layer of fibrous tissue that encased the gland both anterior and posteriorly. Surprisingly, the studies since Cooper [1] did not refer to theses layers as the Anterior and Posterior Lamellae. The first study to investigate the superficial fascial system of the body occurred 150 years after Cooper's work [40]. The study included both cadaveric and surgical dissections of the fascial structure of 12 fresh and embalmed cadavers and 20 body contour patients (patients undergoing cosmetic surgery). The dissections were cross-sectional dissections of both the anatomical and surgical specimens in various parts of the body, including the breast. The superficial fascia system of the various parts of the body was found to consistently have a dual layer of connective tissue, which extended from the skin to the underlying muscle fascia [40]. This fascial network was found to be orientated horizontal to the skin, separated by layers of fat of varying thicknesses. The dual layers were joined perpendicularly by interconnecting fibrous septa[40] much like the dual lamellae structure of the breast described by Cooper [1]. Lockwood et. al (1991)[40] suggested the primary function of the fascial network was to encase, support and shape the subcutaneous fat of the body and anchor it to the underlying tissues. Although the fascial system of the breast was also described to have this dual layer fascial organisation, [40] the study did not compare or refer this organisation to Cooper's [1] descriptions of the breast and therefore these dual fascial layers were not referred to as the Anterior Lamellae and Posterior Lamellae. Nor were the perpendicular interconnecting fibred referred to as Cooper's [1] Anterior and Posterior Extensions despite the similarities between Lockwood's and Cooper's [1] findings. Consequently this nomenclature did not allow the descriptions to be translated to anatomical descriptions of the breast in anatomy textbooks despite the verification of Cooper's findings.

Although Cooper [1] stated that the Anterior and Posterior Lamellae encased the mammary gland, he did not consider them to act as a capsule to the breast. In contrast with Cooper [1], three dissection studies[41, 42, 45] have described a distinct breast capsule. Unfortunately, none of these studies related the anterior and posterior breast capsules that they found to the Anterior and Posterior Lamellae described by Cooper. Riggio et. al. (2000)[45] investigated the fibro-adipose structure of six cadavers (three male and three female) and 21 plastic surgery patients. The study[45] reported that superficial fascial layers around the breast did not split to encapsulate the breast but rather sat posterior to a breast capsule. A second study [42] conducted on eight fresh female cadavers, two embalmed female and two fresh male cadavers (age 77±15 years), also described two membranes that encapsulated the mammary gland anteriorly and posteriorly, with interconnections between the capsule walls consistent with Cooper's [1] descriptions of the Posterior Extensions of the Anterior Lamellae and the Anterior extensions of the Posterior Lamellae [42]. Unfortunately they also did not use any of Cooper's [1] terminology for these fascial structures and re-named all of Coopers[1] extensions "Suspensory Ligaments". They did extend Coopers work however by describing the fascial structure beyond the boarder of the gland. They described the fascia of the anterior capsule to fuse with the superficial fascial system in the inferior aspect of the breast beyond the boarder of the gland [42]. Neither study [42, 45] however provided any quantitative data to support their descriptions of the fibro-adipose structures of the breast.

More detail of the fibro-adipose structure beyond the perimeter of the gland inferiorly was provided in an anatomical study conducted in 2014 on 40 cadavers using blunt and sharp dissections, with sagittal cross sections [41]. This study also described a well-defined breast capsule superior to the level of the fourth rib. Inferior to the 4th rib the capsule became obscured by glandular tissue and ducts forming the nipple-areola complex, a system which was described to have direct dermal insertions of ligamentous tissue, anchoring the breast to the 5th rib [41]. Unfortunately, the study did not comment on the organisation of the breast capsule or fibro-adipose structure beyond the glandular tissue in the medial, lateral and superior breast regions. The study did however relate the anterior and posterior breast capsule they found to Cooper's [1] Anterior Lamella, and Posterior Lamellae. The posterior capsule was described to function as a gliding plane between the pectoral fascia and the breast [41]. Furthermore, they did not specify the location where the fascial layers split to encase the gland, refer to Cooper's [1] Extensions of the breast or provide quantitative data to verify their descriptions.

A limitation of most previous studies describing the fascial structure of the breast has been the direct link of the breast capsule/Lamellae to the gland when the gland does not cover the entire surface area of the breast. Therefore the fibro-adipose structure beyond the gland has not been described and there has been little anatomical quantitative data collected on the surface area of the breast that the gland actually covers. Further research is therefore required to investigate and quantify the fascial structure beyond the gland.

In vivo studies support the notion of an anterior and posterior breast capsule (Cooper's [1] Anterior and Posterior Lamellae), with ultrasound studies finding the breast parenchyma to be enveloped by a superficial and deep fascial plane [70, 71]. Pearson et al [71] described the connective tissue and fascia as bright reflectors what run between and interconnect the fascial planes, encasing the adipose tissue [70, 71].

Unfortunately the visualisation of the detail of the fibro-adipose structure with ultrasound is limited due to the highly reflective nature of the fascial tissue. Therefore, although these studies confirm the existence of the fibro-adipose structure within a fibrous capsule, they provide little detail of its structure.

Due to the inconsistencies in the nomenclature of the fascial tissue laying anterior and posterior to the gland (Anterior/Posterior breast capsule versus Anterior/Posterior Lamellae), the lack of information about the fibro-adipose structure beyond the borders of the gland and the limited detail of the fibro-adipose structure that can be visualised *in vivo* by technology-based research, further anatomical research of the fibro-adipose tissue is required.

Extensions of the Anterior and Posterior Lamellae

Cooper[1] described multiple fascial connections extending perpendicular from the Anterior and Posterior Lamellae encasing the gland which he named the “Anterior and Posterior Extensions” (a total of four sets of attachments). Unfortunately he provided no quantitative data of the number, length or thickness of these fibrous extensions. Extending from the Anterior Lamellae anteriorly he described these fascial bands to connect the superficial breast “capsule” to the dermis, encasing much of the adipose tissue within the breast. He called these the Anterior Extensions of the Anterior Lamellae; however he also referred to these bands as “Ligamenta Suspensoria”. Cooper[1] stated that they functioned to suspend the breast from the skin. Although Cooper [1] provided drawings of these lamellae, he only mentioned the length of the “ligamenta suspensoria” once within his extensive work, in one figure caption, stating it ranged 0.5-2.5 cm[72] (Figure 2). Unfortunately no detail was provided of the methods used to measure the length of the “Ligamenta Suspensoria” nor was any detail included on the number of measurements recorded at the time.

No studies since Cooper [1] have specifically investigated to the Anterior Extensions of the Anterior Lamellae however many studies have described short fibrous extensions connecting the anterior gland to the dermis in their illustrations[36-39]. Unfortunately these have not been labelled specifically but rather all of the fascial within the breast has been referred to as a group as Cooper's [1] Ligaments (or Suspensory ligaments). This has led to confusion in the location and existence of the fibro-adipose structure within the breast. Furthermore, there is no quantitative data amongst the dissection studies to clarify the location, structure and relative distance that each set of extensions spans to verify the work of Cooper [1].

Five dissection studies [36-39] have mentioned the "Suspensory ligaments" although they were not specifically investigating them or the fibro-adipose structure of the breast. One study[45] described Cooper's [1] Anterior Extensions of the Anterior Lamellae, however named them "Coopers Ligaments". Similar to the Anterior Extensions of the Anterior Lamellae, Riggio et. al. (2000)[45] described "Coopers Ligaments" connected the superficial fascial system to the skin directly. A dense bundle of fibres spanning anteriorly from the superficial breast to the dermis was also identified by Komiya et. al. (2015) [39] in their dissections of the nipple-areolar complex (n=5 cadaveric breasts). The authors suggested that the purpose of this tissue is to act as a site for peri-areolar attachment in surgery [39].

Extending posteriorly from the Posterior Lamellae Cooper described fibrous bands which connected the breast to the chest wall via the Superficial Pectoral Fascia. He named these the Posterior Extensions of the Posterior Lamella. Cooper [1] suggested that this complex fascial arrangement encasing the adipose tissue was essential to allow the breast to swing in space and yield to pressure and violence[1].

Cardoso et al. (2015) [36] in a study using liposuction of adipose tissue described the same fibrous structure in 14 breasts (age 66.7; range: 39-85 years) attaching the breast to the chest wall but he did not refer to them as the Posterior Extensions of the Posterior Lamellae. The Posterior Extensions of the Posterior Lamellae were also briefly described, in a study of the superficial pectoral fascia, as weak and easy to bluntly dissect [37]. Though again they were not named or references to Cooper's Posterior Extensions of the Posterior Lamellae. Cooper also described perpendicular fascial bands running from the Lamellae that penetrated the gland and helped to provide structure to it. Extending posteriorly from the Anterior Lamellae he named them the Posterior Extensions of the Anterior Lamellae and extending anteriorly from the Posterior Lamellae he named them the Anterior Extensions of the Anterior Lamellae. These fascial extensions were described to interconnect within the gland and functioned to hold the glandular tissue together. These have een identified in dissection studies since Cooper as part of the gross anatomy of the gland. They have been referred to as fibro-glandular tissue and considered to be part of the support structure for the gland rather than part of the fibro-adipose tissue of the breast.

Cooper's [1] Extensions were also identified in a study that compared the ultrasound image of a coronal breast slice to the anatomy of the same breast slice. Although these studies confirm that the four sets of fibrous tissue extensions exists, further anatomical dissection research specifically investigating these extensions is required that collates the various names of these tissues to allow the fibrous tissues within the breast to be clearly understood and taught.

Not only have the names of the fascial interconnections of the breast varied in the published literature, so has their physical dimensions. They have been described to be "large, strong and numerous" structures, "tentacle like qualities, extending in all directions from the anterior fascia layer (lamellae) to the skin with no discernible organised structure” [73, 74], and “fibrous bands of connective tissues that travel throughout the breast and intersect with the dermis perpendicularly” [68]. This confusion in the physical structure of these fascial tissues within the breast has translated into anatomical textbooks creating confusion regarding the structure of the fibro-adipose anatomy of the breast. Further research is therefore required to provide quantitative data to support the descriptions of the fibro-adipose structure of the breast so that the breast anatomy can be consistently and accurately taught and illustrated in anatomical textbooks

Fibro-Adipose Pockets Anterior and Posterior to the Gland

Cooper [1] stated that the Anterior Extensions of the Anterior Lamella and the Posterior Extensions of the Posterior Lamella formed multiple fascial pockets, which encased the adipose lobules and organised the adipose tissue into compartments located anterior and posterior to the gland [1]. Unfortunately Cooper [1] provided no quantitative data regarding the number or size of the adipose tissue pockets in each region, only that the adipose tissue pockets located anterior to the gland were larger and less numerous than those located posterior to the gland. Cooper [1] also described the presence of additional adipose tissue pockets which were even fewer in number and size which were embedded between the lobules of the mammary gland. These may have been formed by the Posterior Extensions of the Anterior Lamella and the Anterior Extensions of the Posterior Lamella; however this was not specifically dissected.

No previously published anatomical or surgical studies were found that have investigated the fibro-adipose pocket structure of the breast to verify or dispute Cooper’s[1] descriptions. Some sagittal illustrations of the breast have illustrated adipose tissue confined within the suspensory ligaments however a pocket-like fibro-adipose structure has not been described. Lockwood et. al (1991) [40] and Scarpa (1809)[75] in their studies of the superficial fascial system of the whole body did describe the adipose tissues to be organised within a lobule arraignment with fascial layers, however they did not specifically investigate the breast region. Surprisingly, no published study was found that provided any quantitative data on the relative size or number of the adipose tissue lobules within the breast.

Evidence of Cooper's [1] fibro-adipose pocket arrangement has been provided in *in vivo* studies (using MRI and Ultrasound)[70, 71]. They described large adipose tissue lobules located anterior to the gland and smaller ones located posterior to the gland. These studies are limited however in the level of detail that they can visualise these structures relative to dissection studies due to the highly reflective nature of the facial tissue within the breast. Further anatomical dissection research is therefore required to verify the existence of and provide quantitative data on the number and size of the fibro-adipose pocket structure of the breast. This would allow clear and consistent descriptions of the fibro-adipose structure of the breast to be translated in to anatomical textbooks and anatomical education of breast anatomy.

1.2.2 Fibro-Adipose Structure (Textbook review)

Anterior and Posterior Lamellae

The majority of the 31 anatomical textbooks examined had little or no written text relating to the fibro-adipose structure of the breast (Table 2). Of the 31 textbooks, 18 textbooks included a single figure (illustration) of the breast, which illustrated some detail of the fibro-adipose structure either in the coronal or sagittal plane (Table 2, Figure 3). However, none of these illustrations labelled the Anterior or Posterior Lamellae and no detail of the fibro-adipose structure was included in any of the figure captions or associated written text.

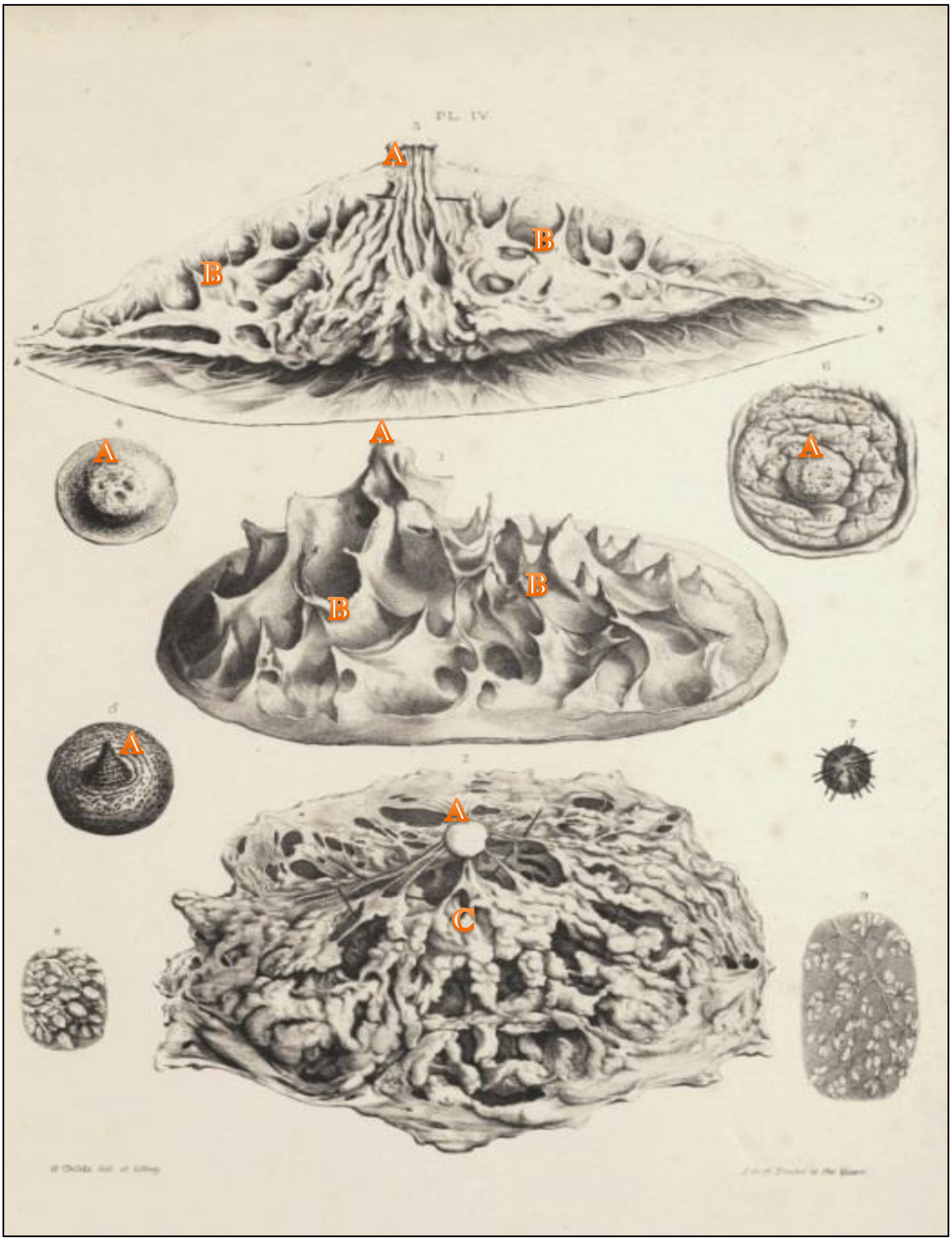

Figure 2: Ligamenta Suspensoria (fat and skin removed) A) Nipple areola complex, B) Ligamenta Suspensoria, C) Glandular tissue (Cooper 1840)[1].

[1]Cooper did not label any of the figures

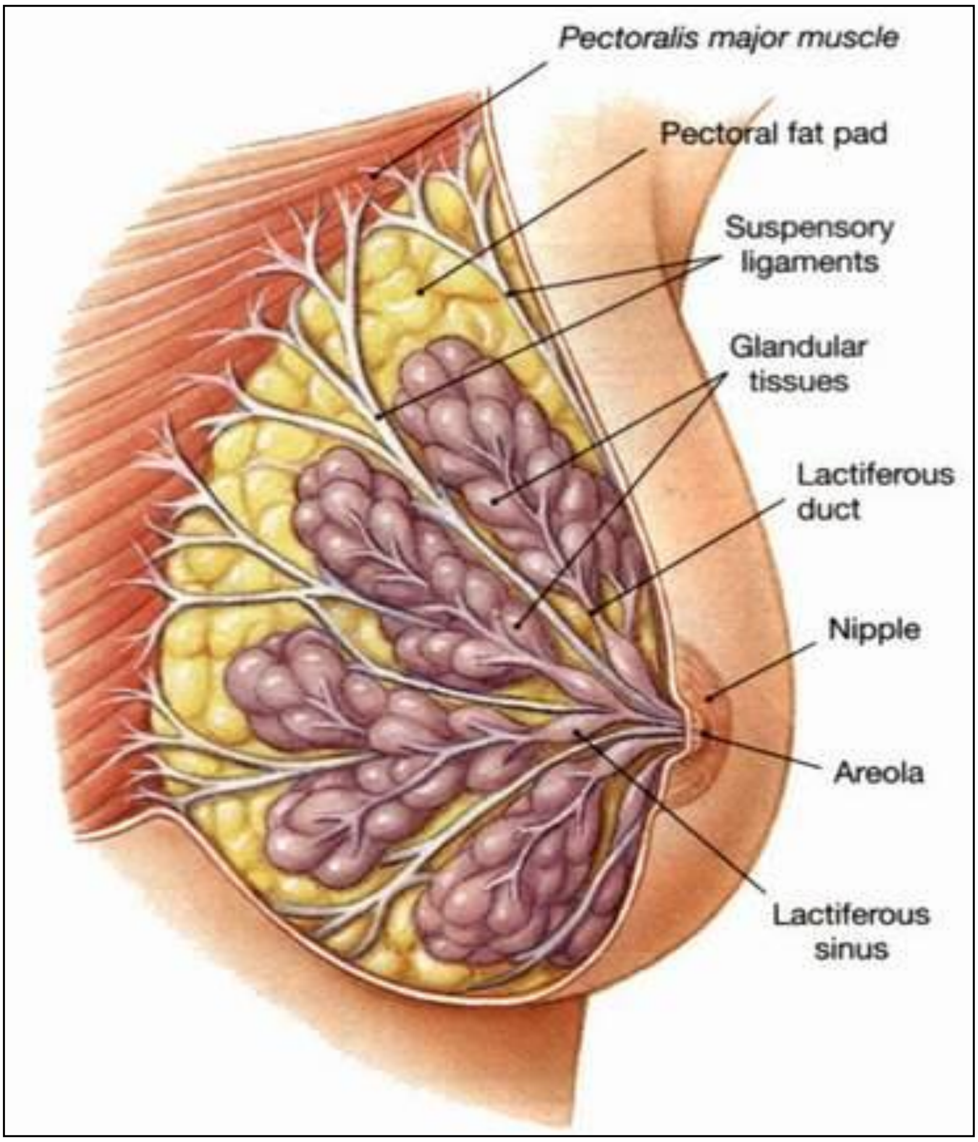

Figure 3 Example of textbook representation of the fibro-adipose sturucture of the breast (Moore, Agur et. al. (2015)[21])

Extensions of the Anterior and Posterior Lamellae

Although Cooper's [1] extensions from the Anterior or Posterior Lamellae appeared in both the anatomical illustrations and the associated written text, there was considerable variation in what they were called, their physical arrangement within the breast and in their location (their origin and insertion). Rather than Anterior or Posterior Extensions, the fascial tissue of the whole breast was generally referred to as "Coopers Ligaments" or "Suspensory Ligaments". These inconsistent terminologies also create further confusion as Cooper's [1] used the name "suspensory ligaments" to specifically refer to the Anterior Extensions of the Anterior Lamellae.

The arrangement and location of the Anterior or Posterior Extensions in the 19 textbooks that either mentioned or illustrated "Coopers Ligaments" or "suspensory ligaments" (Table 2), was also inconsistent. Seven textbooks displayed the ligaments to span from the dermis to the glandular tissue [13-17, 29, 32] while eight other textbooks displayed them spanning from the dermis to the superficial pectoral fascia [6, 10, 11, 21, 22, 26, 30, 31]. The four remaining textbooks did not clearly show where the ligaments spanned from or connected to. Consequently, as the naming, location and relative length difference of these ligaments within the breast in anatomical textbooks is inconsistent and unclear, the current status of university-based anatomical education and the understanding of the fibro-adipose structure of the breast of medical and allied health graduates is generally poor.

Fibro-Adipose Pockets Anterior and Posterior to the Gland

The fibro-adipose pocket arrangement of the breast was illustrated in a coronal illustration in only two of the 31 textbooks included in the summary [4, 16]. Neither of these textbooks had detailed accompanying text describing this structure. Only four textbooks made any reference to an organised fibro-adipose structure (Table 2) but no pocket-like organisation was described [4, 16, 19, 22]. No clear organisational structure of the fibro-adipose structure was evident in the coronal images of the other textbooks and no quantitative data or cited references were provided to support the descriptions included. The lack of any quantitative data on the fibro-adipose pocket structure within the breast may assist to explain why descriptions have not been translated into anatomical textbooks and why such variation exists in the anatomical illustrations within anatomical texts.

1.2.3 Fibro-Glandular Structure (Literature Review)

The fibro-glandular structure of the breast was extensively investigated by Cooper [1] by injecting mercury into the glandular tissue to trace its pathway through the gland. He described the mammary gland to have 7-10 separate segments, up to 12 lactiferous tubes and 22 orifices, which all opened at the nipple[1]. His descriptions detailed the anatomy of the lobule arrangement, the ductal structure, and the ampulla and included quantitative data.

Cooper's [1] description of the organisation of the fibro-glandular structure into lobes, lobules, ducts, ampulla and sinuses all opening at the nipple has been verified by quantitative data from dissection studies [38, 44] and literature review studies [44, 74, 76-80], which have been consistent with each other. These studies consistently report the mammary gland to have 15-20 separate segments (lobes) (greater than Cooper's [1] 7-10 separate segments), which consistent with Cooper, disperse radially from the nipple [74, 76-80]. The methodology from technology-based studies (QT Ultrasound scan) of the anatomical structure of the gland, viewed from a coronal breast slice has also been verified and consistent with dissection studies of the same slice of breast tissue [38]. These studies have also linked the gross anatomy of the fibro-glandular tissue to structural changes in the tissue that can coincide with pathologies of the fibro-glandular tissue. The consistency of the anatomical descriptions of the gross anatomy of the fibro-glandular tissue, supported by quantitative data has enabled the detail of the gross anatomy of the fibro-glandular tissue to be translated into anatomical illustrations and written text within both the published literature and anatomical textbooks. In contrast to illustrations of the fibro-adipose structure of the breast, these anatomical illustrations have labels, associated figure captions and associated written text.

1.2.4 Fibro-Glandular Structure (Textbook review)

The gross anatomy of the fibro-glandular tissue and its organisation regarding the connection of lobes, lobules and ducts opening at the nipple structure was described in 24 of the 31 textbooks included within the textbook review (Table 2). These descriptions were consistent with each other and the descriptions of Cooper[1] and more recent anatomical studies. Minor variations were found regarding the classification of the mammary gland, the number of lobes and the location of the glandular tissue. Twelve anatomical texts described the mammary gland as a modified sweat gland [7, 13, 14, 17, 19, 21-23, 26, 27, 30, 31]; 21 describe the gland to consist of 15-20 segments (lobes) [2, 4-7, 12, 13, 15-17, 19-22, 26-28, 30-32]; and nine (9) texts described the mammary gland tissue to be separated by a fibrous septa [4-6, 9, 17, 20, 26, 28].

The location of the fibro-glandular tissue within the breast had the greatest variation amongst the textbook illustrations, mainly evident from the sagittal view of the breast. Eight textbooks illustrated the fibro-glandular tissue to be encased between two layers of fibro-adipose tissue [5, 13, 14, 26, 29-32], five displayed it directly anterior to the superficial pectoral fascia (with only fibro-adipose tissue anterior) [10, 11, 16, 17], and four had no discernible difference between the location of the fibro-adipose and fibro-glandular tissues of the breast [4, 6, 21, 22]. There was considerable variation noted within the anatomical illustration amongst the textbooks regarding the relative percentage of the total composition of the breast with no two images comparable.

The gross anatomy of the fibro-glandular tissue has been consistently described and included within the majority of anatomical textbooks, supported by quantitative data. However, variation was found in the location of the glandular tissue relative to the fibro-adipose structure within the breast, the surface area of the breast that the gland covers and the percentage composition of the breast that is fibro-glandular tissue. Further anatomical research is therefore required to provide data to verify these anatomical descriptions in terms of the location, surface area and contribution of the fibro-glandular tissue to the composition of the breast.

1.3 Attachment of the breast to the chest wall

1.3.1 Regional Anatomy (Literature Review)

Cooper [1] described the breast to sit on the anterior chest wall between ribs 2-7 in the superior-inferior directions, and between the sternum and mid-axillary line in the medial-lateral direction. He stated that the posterior aspect of the breast sat on the Pectoralis major muscle and the lateral edge of the breast was in contact with the Serratus Anterior and External Oblique muscles [1]. Unfortunately, he did not provide data on the number or age of the female cadavers this described location was based on.

Of the 17 cadaveric dissection studies of the breast (Table 3), 10 studies commented on the regional anatomy of the breast [34-37, 40-43, 45, 48] which were all consistent with Cooper's [1] descriptions. The only difference was in the addition of another muscle having contact with the posterior wall of the breast, Rectus Abdominal muscle [37]. Although these studies verify the location and regional anatomy of the breast on a greater number of cadavers (over 100 cadavers between the 10 studies) none of the studies quantified the percentage of the 'footprint' of the breast that each muscle has contact with. Therefore, no anatomical data exists as evidence on the percentage contribution of the superficial chest wall muscles (Pectoralis Major, Serratus Anterior, External Oblique and Rectus Abdominus muscles) to the footprint of the breast.

The perimeter of the breast has also not been quantified in terms of its length by Cooper [1] or any dissection study since. Some data has been collected through *in vivo* studies on the length of the inframammary fold, which was measured to be 10-12cm long [77, 80] and on the horizontal diameter of the breast, which has been found to be 14.3±1.4cm (range: 8.5–23.5cm [53]). Although these measurements provide some information of the perimeter of the breast, further quantitative data is required that specifically measures the perimeter of the breast in relation to the surface area and volume of the breast.

1.3.2 Regional Anatomy (Textbook review)

Sixteen textbooks included descriptions of the regional anatomy of the breast [2, 4, 6, 7, 9-14, 19, 21-23, 27], which again were consistent Cooper[1] and the published literature [1, 68, 74, 77-80]. Of the 26 textbooks that contained coronal images, the regional anatomy was consistently illustrated in 11 textbooks (Figure 7)[2, 4, 6, 7, 9, 14, 15, 22, 24, 25, 32]. Only one textbook illustrated the regional anatomy of the breast with a sagittal illustration [6]. The regional anatomy of the breast was consistent within the coronal and sagittal illustrations regarding the muscles that the posterior wall of the breast was in contact with (Pectoralis Major, Serratus Anterior, External Oblique and Rectus Abdominus), however no quantitative data was provided however to verify the location of the breast, and the percentage of area that each muscle occupies within the perimeter.

Within the written text however, there was some inconsistency regarding the muscles that the posterior wall of the breast was in contact with. Nineteen of the 31 textbooks (Table 2) described the muscles which were located directly posterior to the breast [2, 5-7, 9-12, 19-22, 26-28, 30-32]. Pectoralis major muscle was consistently described to be in contact with the posterior wall of the breast, however variation existed regarding Serratus Anterior, External Oblique and Rectus Abdominus. Serratus Anterior muscle was included in six textbooks [9, 10, 13, 14, 21, 22], External Oblique in 13 [2, 4-6, 10, 11, 13, 14, 21, 22, 30-32] and Rectus Abdominus muscle in 14 [2, 4, 7, 9-11, 21-23, 26-28, 30, 31]. None of the textbooks described the relative percentage each muscle contributed to the posterior aspect of the breast. The results of this textbook review confirm that further anatomical research is required that measure the percentage contribution of each of the muscles to the posterior wall of the breast to update anatomical textbooks and anatomical education.

1.3.3 Attachment of the breast to the chest wall (Literature Review)

Cooper [1] stated that the breast was anchored to the adjacent chest wall by the Posterior Extensions of the Posterior Lamella, which penetrated the superficial fascia of the Pectoralis Major muscle [1]. He did not however, describe the number, length or width of the Posterior Extensions, nor did he give any indication of the strength of these attachments. The Posterior Extensions were the only attachment of the breast to the chest wall described by Cooper, he did not describe any perimeter attachment. Cooper stated that the Posterior Extensions were designed to allow the breast to “swing from the chest wall”[1].

The ***posterior attachment*** of the breast to the chest wall via the Posterior Extensions of the Posterior Lamellae was verified by Jinde et. al. (2006) [37] in a study investigating the superficial pectoral fascia of 30 cadaveric breast specimens (ages unknown). The Posterior Extensions were described to consist of many thin, fibrous bundles in the superior region of the pectoral fascia which connected to the Posterior Lamellae of the breast [37]. The study reported that these fibrous bundles were particularly evident around the level of the 4th rib and formed a layer of filmy areola tissue, which allowed the breast to move freely on the fascia of pectoralis muscle fascia (Figure 4) [37]. In contrast to Cooper however, Jinde et. al. (2006) [37] stated that the Posterior Extensions of the Posterior Lamella did little to anchor the breast to the chest wall and that the breast was attached to the chest wall along its inferior perimeter and through a horizontal septum at the level of the 4th rib. Unfortunately, the study provided no data to support this claim. Three other dissection studies [36, 41, 48] published since have agreed with Jinde et. al. (2006) [37] rather than Cooper[1] that the Posterior Extensions of the Posterior Lamellae provide only a weak connection of the breast to the chest wall, as they were easily broken with blunt dissection.

The notion of a ***horizontal attachment*** through the breast connecting the breast to the trunk was also reported by Wűringer et. al. (1998)[48] in their cadaveric dissection study of 28 fresh cadaveric specimens (68-92 years)[48]. In contrast to Jinde et. al. (2006) [37] however, the horizontal septum was described to anchor to the ***medial and lateral borders (perimeter)*** of the breast rather than through the inferior perimeter. In all 28 cadavers dissected, the authors consistently found medial and lateral ligament extensions of fascial tissue which attached the breast onto the chest wall, originating from a horizontal septum that divided the breast into superior 2/3 and inferior 1/3 (Figure 5) [48]. The medial and lateral extensions crossed from the retro-mammary space of the breast to the Anterior Lamella and curved upwards as vertical ligaments. The medial and lateral attachments were described to have superficial and deep attachments over their entire length. The deep origin of the medial ligament was the sternum, level with the 5th rib and the superficial attachment was the skin. The deep and superficial origins of the lateral ligament split from the lateral edge of pectoralis minor fascia [48].

A horizontal attachment was also reported by Cardoso et. al. (2015)[36] in an anatomical dissection study of 14 breasts (age 66.7; range: 39-85 years). The adipose tissue of the breast was removed using liposuction to reveal a horizontal attachment of the breast to the chest wall and two additional attachments which arose from the 5th rib, a lateral perimeter attachment and the inferior perimeter attachment. The lateral perimeter attachment was seen to run in an oblique direction parallel to the lateral boarder of the pectoralis major muscle, 2-3cm wide and 7-13cm long.

Medial and lateral perimeter attachments were also found in Matousek et. al (2014)[41] dissection study of 40 cadavers however these were in conjunction with a superior and inferior perimeter attachment. Matousek et. al (2014)[41] named this medial attachment the Medial Sternal Ligaments and reported them to be short, strong ligaments, running horizontally from the medial aspect of the breast to the sternum, forming a very firm zone of adherence of the breast to the chest wall and contained very little fat and no glandular content. The authors [41]suggested that the length of these medial ligaments varied with age, appearing longer in breasts that displayed a large level of ptosis. The authors named the lateral ligaments the Pectoralis Minor Suspensory Ligament and the Lateral Fascia Confluence. The Lateral Fascia Confluence was described to be a fusion of superficial fascias covering the Serratus Anterior, Pectoralis Major and External Oblique muscles of the anterior/lateral aspect of the chest wall and was a superior continuation of the curve of the inframammary fold. The superior extension of the Lateral Fascia Confluence was described to extend into the axilla where it was named the Pectoralis Minor Suspensory Ligament, which links the clavi-pectoral fascia to the axillary skin.

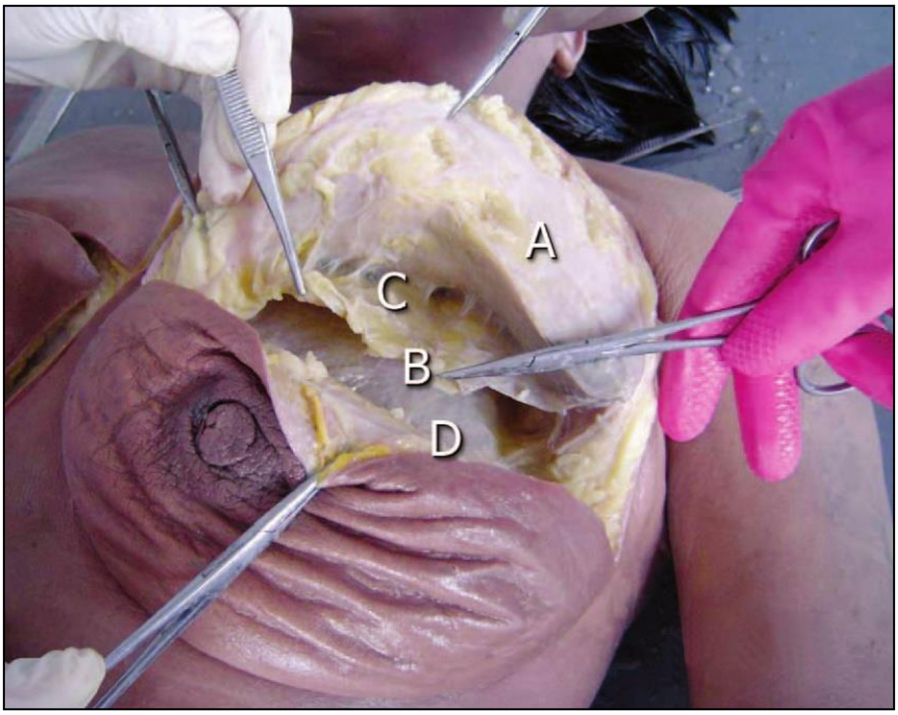

Figure 4: Cadaveric breast dissection showing A) Glandular tissue, B) Superficial pectoral fascia, C) Posterior Extensions of the Posterior Lamellae, D) Pectoralis major muscle (Jinde et. al 2006).

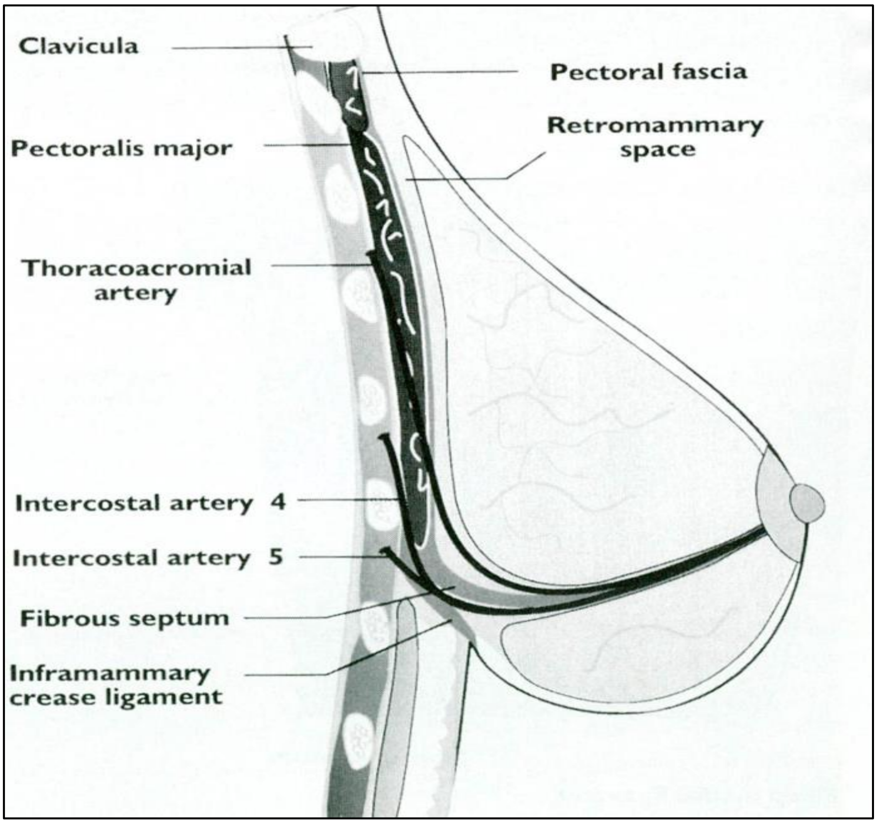

Figure 5: Horizontal septum spanning medio-laterally from the 5th rib to the nipple (Wűringer et. al. 1998).

There has been some debate in the literature regarding the ***inferior perimeter*** attachment of the breast to the chest wall, or the inframammary fold. Cooper [1] did not specifically mention the inframammary fold, however 10 studies since Cooper [1, 34-36, 41-43, 45, 48, 81, 82] (2 surgical and eight cadaveric) have investigated it. The 10 studies agreed that the inframammary fold formed the inferior perimeter attachment of the breast where fibrous tissue connected the chest wall with the dermis at the lower curvature of the breast [34, 43, 83] (Figure 6).

Although there is consensus on the presence of the inframammary fold, conflict exists over the orientation and attachment of these fibres within the inframammary fold. Three studies (two cadaver and one surgical) describe fibres orientated in the sagittal plane, running between the superficial pectoral fascia level with the 5/6th rib and inserting into the deep dermis [34, 45, 81]. Another three studies (one surgical and two cadaver) [35, 48, 82] describe transverse orientated fibres spanning from the mediolateral width of the inframammary fold region, connecting into the fascia of the sternum [45, 82].

There is some consensus that fibres within the inframammary fold attach to the dermis. Lockwood et. al. (1991)[40], in his study of the superficial fascial system of the body, described the skin to have zones of adherence, where the topography of the skin changed due to adherence of the skin to the underlying fascia to the dermis, the study described the inframammary fold at the level of the 5th rib to be one of these zones. One of the most recent cadaveric studies of 40 cadavers, Matousek et.al (2014)[41] verified Lockwood’s dual ligament system at the inframammary fold, which provided support and anchorage to the dermis above and below the region of the inframammary fold. A study of 74 breast reconstruction patients [67] also reported the ligamentous part of the inframammary fold connected the deep dermis to the superficial pectoral fascia at the level of the 5th/6th rib. Studies report that surgical re-connection of these attachments of the inframammary fold produced a high level of patient satisfaction both immediately post-operatively and one year after the surgery compared to surgeries without these attachments. The authors stated that the collaboration of the ligamentous tissue of the inframammary fold and its dermal connections were fundamental to support the mass and shape of the breast [67].

The location of the inframammary fold or inferior perimeter attachment has been consistently described to be level with the 4th or 5th rib and to extend from the parasternum to mid-axillary line and to have a curved or “crescent” shape with an approximate diameter of 10-12 cm [77, 80, 84]. Cardoso et. al. (2015)[36] described the inferior attachment to be level with the 5th rib in 12 of the 14 breasts and below the 5th rib on the other two breasts. Rather than a bony attachment, Nanigian et. al. (2007)[43] found a fascial attachment of the inframammary fold to the adjacent to the chest wall muscles from there study where methylene blue was used to tattoo the inframammary fold to the level of the chest wall muscles in 20 cadavers (age: 74-95 years). Once the breast was dissected, the methylene blue stain was consistently seen inferior to the inferior margin of the Pectoralis Major muscle in all cadavers. The authors explain the location of the inframammary fold to have a relationship with the Pectoralis Major muscle rather than the ribs. The study provided quantitative data regarding the location of the attachment however it did not comment on the orientation of the fibres [43].

The inframammary fold has been consistently found in anatomical and histological studies to contain collagen and elastin fibres and as such is referred to as a ligamentous structure [34-36, 41-43, 45, 48, 81, 82]. Matousek et al (2014)[41] in their dissection study of 40 cadavers described the Triangular Fascial Condensation in the inferior aspect of the breast. The Triangular Fascial Condensation was described to consist of non-ligamentous fibres running oblique and inferior from the periosteum of the 5th rib, which fanned out in a triangular fashion and inserted into the dermis. Horizontal fibres were also identified running between the deep Rectus Abdominal fascia and the dermis.

Regardless of the precise location, orientation or histological aspects of the inframammary fold, breast surgeons consider it to be very influential to breast ptosis, the shape of the inferior pole of the breast and the position of the breast mound on the chest wall. As such, the inframammary fold has also been researched extensively in *in vivo* studies [67, 85], as preservation of the inframammary fold in surgery is essential to maintain the natural appearance and the aesthetics of a reconstructed breast [35, 67].

Only one published study was found that described the superior perimeter attachment of the breast to the chest wall [41]. The dissection study of 40 female cadavers using blunt and sharp dissections described the attachment of the breast to the chest wall along its entire perimeter. The authors also reported the perimeter to be the primary anchor of the breast to the chest wall. The authors identified numerous ligamentous attachments along the entire perimeter of the breast in 1.5-3cm sagittal cross sections of the breast [41]. The study named two superior attachments of the perimeter, the Superficial Clavicular Ligament which connected the superior aspect of the breast to the superficial pectoral fascia and the Deep Clavicular ligaments which was located between the sternal and clavicular heads of the pectoralis major muscle and attach to the inferior portion of the clavicle. The study did not speficiy however the number of cadavers these perimeter attachments were found in or provide any quantitative data of the perimeter attachments..

There is some consensus in the literature that the breast attaches to the chest wall via both the Posterior Extensions of the Posterior Lamellae and along its perimeter, with the perimeter attachment being the stronger anchor of the breast to the chest wall. There is conflict however regarding the structure of the perimeter attachment. That is, whether the perimeter attachment is connected along its entire length or only in some sections. Although there is consensus on the presence and importance of the infra-mammary fold to the shape and support of the breast and its connection to the dermis, there is conflict over its location relative to the ribs and the Pectorals Major muscle. Lastly, although it is agreed that the Inframammary fold consists of collagen and elastin fibres, the structure and naming of the triangular condensation of fascia connecting to the 5th rib is also a new concept. Therefore, further anatomical research is warranted to investigate the inconsistencies in the literature regarding the perimeter attachments of the breasts to the chest wall and the relative strength off the perimeter and Posterior Extensions of the Posterior Lamellae attachments.

1.3.4 Attachment of the breast to the chest wall (Textbook review)

Despite some of the consistencies in the literature regarding the existence of the posterior wall attachments and the perimeter attachments of the breast to the chest wall, very little information of the attachments of the breast to the chest wall has been translated into anatomical textbooks. Eight textbooks show in their illustrations a connection of the breast to the Superficial Pectoral Fascia, however this connection often runs the entire way through the breast to the dermis and cannot therefore be differentiated from the fibro-adipose tissues, particularly the Posterior Extensions of the Posterior Lamellae [6, 10, 11, 21, 22, 26, 30, 31]. Although 22 of the 31 textbooks examined mentioned the fact that the breast does attach to the chest wall, they failed to describe or illustrate just how this occurs (posterior or perimeter attachment). Therefore, anatomical research on the attachments of the breast to the chest wall via both the posterior wall and the perimeter is required to provide a clear and concise description of the attachments that can be translated into anatomy textbooks and anatomical education.

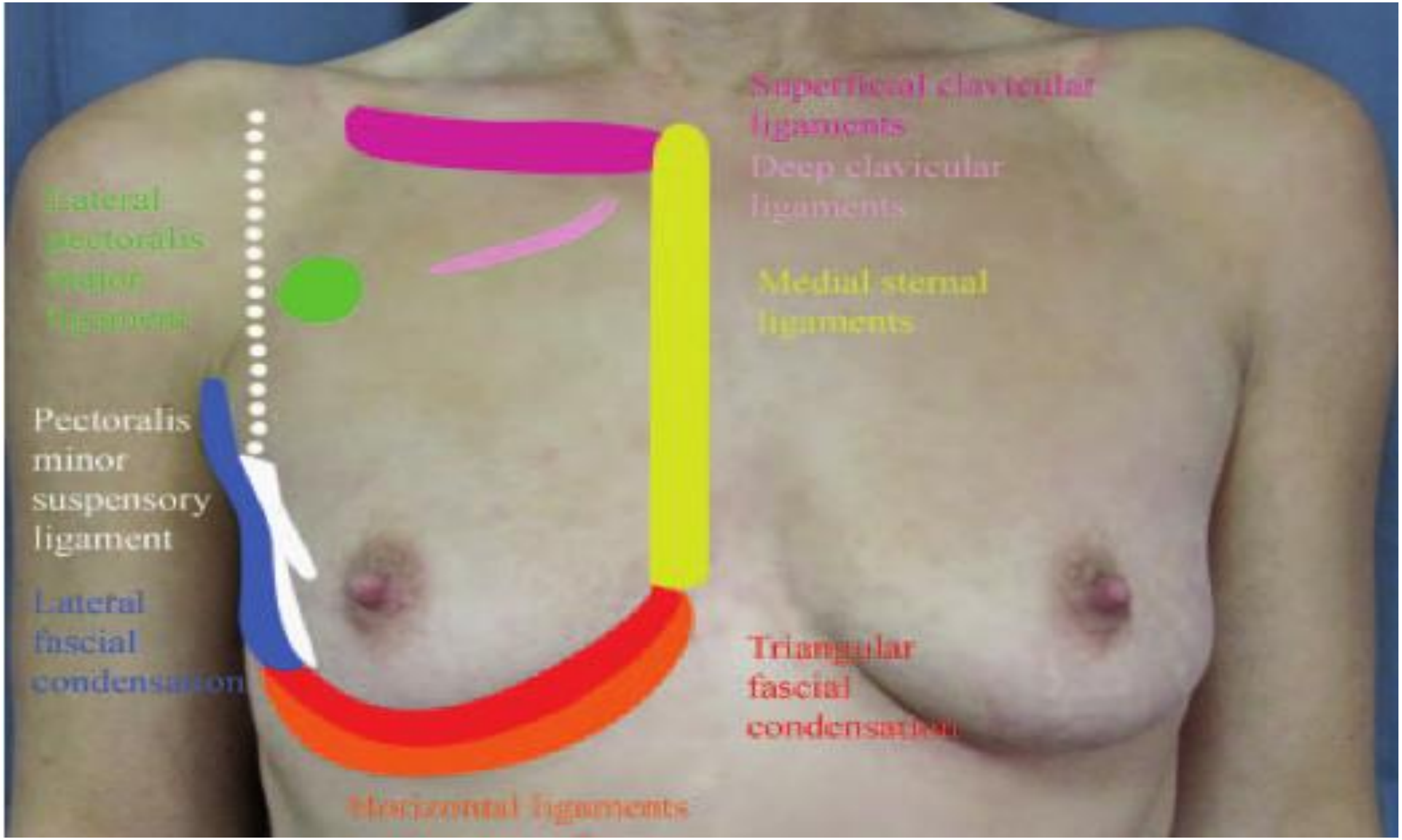

Figure 6: Entire perimeter attachments of the breast to the chest wall (Matousek et. al. 2014).

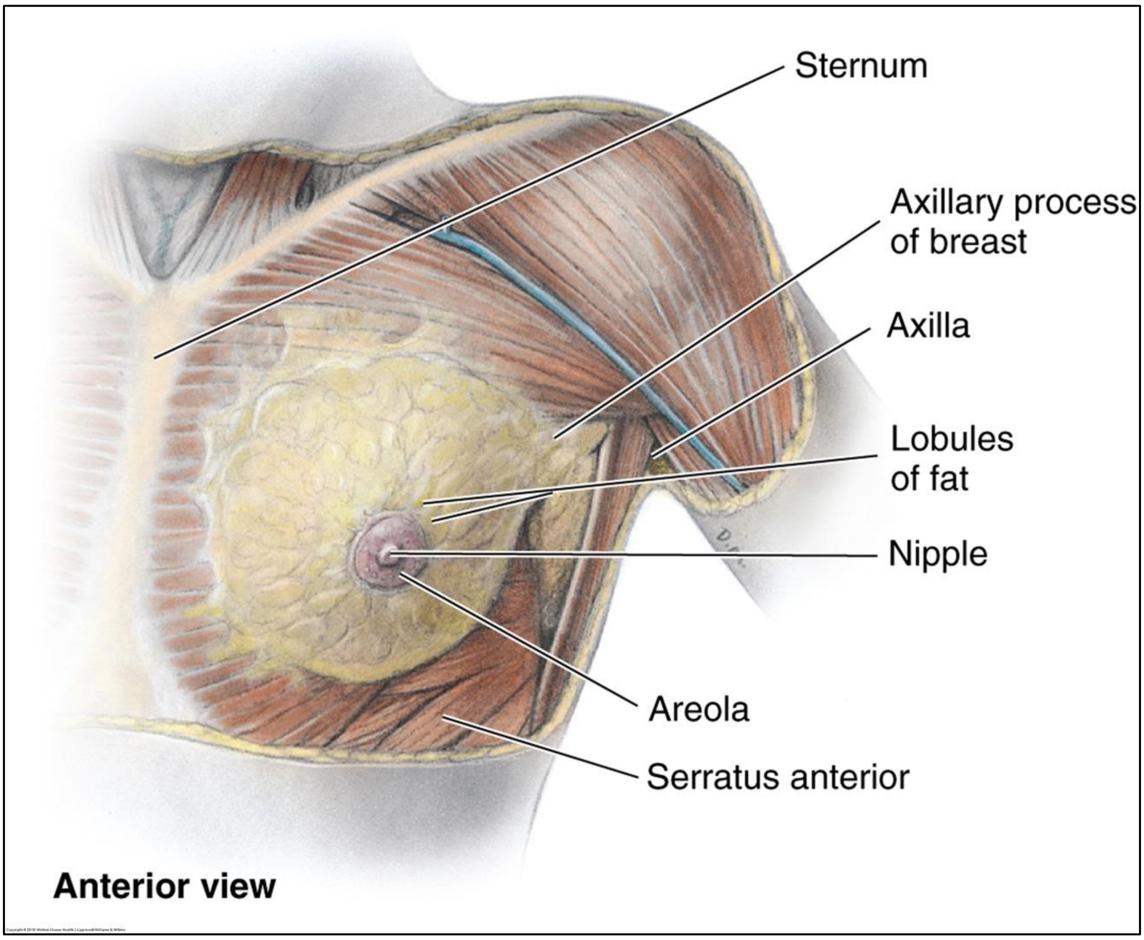

Figure 7 Example of textbook representation of regional anatomy of the breast on the chest wall (Moore, Agur et. al. (2015)[21]

1.4 Literature Summary

This literature review highlights the need for further anatomical dissection research on the gross anatomy the breast, supported by quantitative data due to the dated research, inconsistencies in the literature and the limited detail available in anatomical textbooks currently used for medical, allied health and science students on the structural anatomy of the breast. Specific gaps in the literature were identified on the structural anatomy of the breast regarding:

(i) The composition of cadaveric breasts in terms of the relative percentage of fibro-glandular and fibro-adipose tissue across a spectrum of breast sizes.

(ii) The fibro-adipose structure of the breast in terms of descriptions of its complex structure supported by quantitative data and a collation of the various names used to describe the fascial tissue within this structure.

(iii) Attachments of the breast to the chest wall via the posterior wall and perimeter attachments, supported by quantitative data.

(iv) Regional anatomy of the breast in terms of the percentage contribution of the muscles adjacent to the posterior wall of the breast

Therefore further anatomical dissection research is required to verify the descriptions of Cooper, complementing them with quantitative data. This research could be used as a basis from which concise, evidence-based written text descriptions along with accurate and consistent anatomical illustrations of the breast could be developed supported by quantitative data. The improved understanding and education of the structural anatomy of the breast could contribute positively to both breast surgery outcomes and the diagnosis of pathology.

1.5 Research Aims and Hypothesis

The aim of this study was to provide quantitative data on the gross anatomy of the breast, to provide evidence-based detail for anatomical illustrations and descriptions on the gross anatomy of the breast.

1.5.1 Composition

Aim one: To provide quantitative data of embalmed cadaveric breast size (volume, surface area and mass) and embalmed cadaveric breast composition in terms of percentage mass of fibro-adipose and fibro-glandular tissue. From the interpretations of the literature the following experimental hypotheses were set:

Hypothesis one: Breast size (surface area and volume) will be within the ranges of breast volume and surface area reported in in-vivo studies.

Hypothesis two: The relative percentage mass of the fibro-adipose and fibro-glandular tissue will be consistent amongst the embalmed cadaveric breasts, irrespective of their size (total mass).

1.5.2 Gross Anatomical Structure

Aim two: To investigate and to provide quantitative data on the gross anatomy of the fibro-adipose structure of the breast. In agreement with the descriptions of Cooper the following experimental hypotheses were set:

Hypothesis three: Singular sheets of fascia will be found both anterior (Anterior Lamellae) and posterior to the mammary gland (Posterior Lamellae).

Hypothesis four: Fibrous bands of tissue (Anterior Extensions of the Anterior Lamellae) will be found connecting the Anterior Lamellae to the dermis.

Hypothesis five: Fibrous bands of tissue (Posterior Extensions of the Posterior Lamellae) will be found connecting the Posterior Lamellae to the superficial fascia of the Pectoralis Major muscle.

Hypothesis six: Adipose tissue will be encapsulated within fibrous pockets (fibro-adipose pockets) located both anterior and posterior to the mammary gland.

Hypothesis seven: The number of the fibro-adipose pockets within the breast and the size of the fibro-adipose pockets will be consistent amongst the breasts, irrespective of their size.

Hypothesis eight: The size of the fibro-adipose pockets located anterior to the gland will be larger and less numerous to those located posterior to the gland.

1.5.3 Attachment of the breast to the chest wall

Aim Three: To investigate the regional anatomy and attachment of the breast to the chest wall. From the interpretation basis of the literature and the anatomical descriptions of Cooper the following experimental hypotheses were set:

Hypothesis Nine: The posterior wall of the breast and the perimeter of the breast will have fibrous connections to the superficial muscle fascia of the anterio-lateral aspect of the chest wall.

Hypothesis Ten: The muscles found within the perimeter will include the Pectoralis Major, Rectus Abdominus, Serratus Anterior and External Oblique muscles.

Chapter 2:

Methods

2.1 Experimental Overview

A cadaveric-based investigation was carried out on embalmed female cadaver's post-mortem. The investigation focused on three aspects of the female breast; (i) Composition, (ii) Gross anatomical structure, (iii) Attachments of the breast to the chest wall. Eighteen breasts from nine cadavers were investigated quantitatively and the gross anatomy was described qualitatively using this data. A range of dissection techniques were used including blunt and sharp dissection, coronal and sagittal sectioning, macro and microscopic investigation, photography, tissue weighing and three-dimensional scanning. The breast was investigated in two planes, coronal and sagittal to provide a three dimensional understanding of the structure of the breast.

2.2 Ethics Consideration

All procedures portrayed in this proposal were submitted and approved by the University of Wollongong Human Research Ethics Committee (HE14/001). The University of Wollongong houses a PC2 Anatomy Laboratory where all cadaveric specimens were stored, prepared and dissected according to NSW State Legislation and University policy and guidelines.

2.3 Cadaveric Selection

The breasts of cadavers embalmed, using classical techniques through the left common carotid with a formalin based embalming fluid (Genelyn Anatomical Series NF, GMS Innovation, Australia), were selected, by the chief researcher (KG), from a list of cadavers held in the University of Wollongong Anatomy Laboratory that were approved and consented for anatomical research purposes (n= 24). From this list, specimens were excluded based on the following criteria:

(i) The breast or chest plate had already been dissected, as the integrity of the structure within the breast may have been compromised (n=8)

(ii) Recorded or observable signs of surgical procedures within the breast or chest region, such as mastectomies, lumpectomies and augmentation mammoplasty (n=3)

(iii) Recorded or observable signs of breast shape abnormalities, or abnormalities due to the embalming process, which may skew the accuracy of measurement (n=3)

One cadaver was selected for a reliability study. The nine most recently embalmed cadavers, which were a range of breast sizes, satisfying the inclusion criteria are shown in Figure 8 and Table 4.

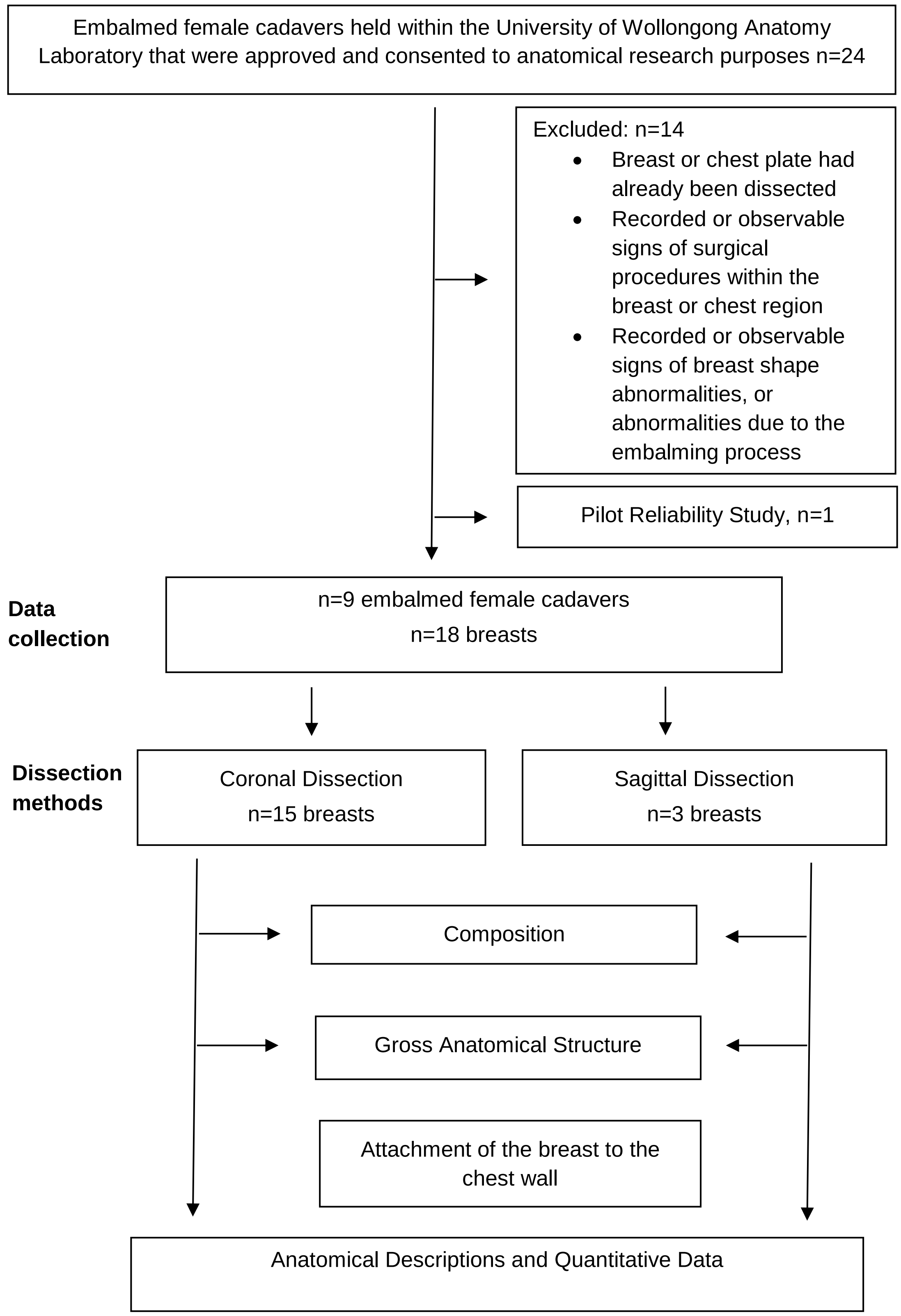

Figure 8: Experimental overview

Table 4: Characteristics and anthropometric data of the cadaveric specimens included in this study.

Breast	Cadaver no.	Age at death (years)	Cause of Death
Coronal 1	203-13	90	Respiratory Tract Infection, Dementia
Sagittal 1			
Coronal 2	197-13	63	Metastatic disease
Sagittal 2			
Coronal 3	188-13	81	Non-Hodgkin’s Lymphoma
Sagittal 3			
Coronal 4	210-14	87	Respiratory Failure, Aspiration pneumonia, Dementia, Chronic frailty
Coronal 5			
Coronal 6	241-15	80	Cardiac arrest, liver cancer, colon cancer
Coronal 7			
Coronal 8	234-14	90	Sepsis, Aspiration pneumonia, Parkinson's disease, Chronic renal failure, Type 2 diabetes
Coronal 9			
Coronal 10	243-15	90	Interventricular hemorrhage, Stroke, Hypertension
Coronal 11			
Coronal 12	244-15	89	Pulmonary oedema, Myocardial infarction/ischemia, Pneumonia, pancreatitis
Coronal 13			
Coronal 14	249-15	71	Ovarian cancer, Pleural effusion and ascites
Coronal 15			
Mean		82	

2.4 Dissection Methods

The gross anatomical structure, composition and the attachments of the breast to the chest wall on the nine female embalmed cadavers (18 breasts) were investigated in both the coronal and sagittal plane. Breasts from both the coronal and sagittal plane were firstly scanned with a handheld three-dimensional white light scanner (Artec™ Eva three-dimensional Scanner, Artec Group, San Jose) and imported to Geomagic (Geomagic Studio® software; version 12; three-dimensional Systems, South Carolina, USA) to calculate the total breast volume and total breast surface area. These values were used to normalise all other measurements. The breasts in the coronal plane were scanned after the removal of skin from the breast region. The breasts in the sagittal plane were scanned prior to the removal of the skin (to ensure no disruption to the tissues when slicing). The sagittal plane breast volumes were divided by a coefficient factor of 1.03, according to the research conducted by Yip et. al. (2012) to allow for comparison between the coronal and sagittal breast scan volumes. The coronal dissections were conducted from a superficial to deep dissection with the breast remaining on the cadaver (n=15) and specific quantitative measurements were taken at each depth of dissection. The sagittal dissections were conducted on different breasts to the coronal dissections (n=3), and required the chest plate to be removed from each cadaver. Different quantitative measurements were recorded in the sagittal plane compared to the coronal plane.

2.4.1 Coronal Dissection Method

With the breast remaining on the embalmed cadaver, the border of the breast was outlined on the skin with a permanent marker (Black 70 Permanent Marker, Pelikan Artline© AUS) (Figure 9a), which was determined through visual inspection and palpation of the borders by the breast by the chief investigator (KG). The perimeter was then marked again by injecting a streak of viscous dye mix of 1:10 India Ink (Black Ink, Windsor and Newton© UK) and ultrasound gel (Aquasomic© 100, Parker Laboratories USA). The injections, made at 2-3 cm intervals around the border, penetrated the perimeter of the breast perpendicular to the skin and injected approximately 1 ml of viscous dye to the entire depth of the breast perimeter to the chest wall. This ensured that the border of the breast was clearly marked for the entire depth of the dissection (Figure 9a).

Skin removal

The skin was then removed with sharp dissection using curved forceps (Swann- Morton, England) and a scalpel handle with a size 10 blade (Swann-Morton, England). Skin incisions were made following the midline of the sternum, at least 2 cm below the inferior boarder of the breast laterally, past the mid-axillary line and superiorly 1 cm above the level of the clavicle and the skin within these incisions was removed. The skin of the nipple and areola region was not removed so that it could be used as a point of reference during the coronal dissection (Figure 9b). The middle of the nipple and the furthermost superior, inferior, medial and lateral points of the breast was then pinned with flag pins (Figure 9c). The pins were also used as landmarks for the three-dimensional scanning analysis. The dissections of the breast were also photographed (Nikon 600D, 200mm Lens) at each stage of the dissection from superficial to deep.

Breast quadrants

The flag pins were then used to join pieces of string that were set up to divide the breast in to four quadrants, superior-medial, superior-lateral, inferior-medial and inferior-lateral. These divisions were used to investigate regional variation in the quantitative measures taken during the coronal dissection. Photographs (Nikon 600D, 200mm Lens) were taken to complement the qualitative descriptions of the breast at this stage (Figure 9c).

Fibro-adipose tissue structure (superficial to the glandular tissue)

The most superficial layer covering the entire breast directly below the skin consisted of a thin layer (1-2 mm) of subcutaneous fat. This was removed from the external surface of the breast with the back edge of curved forceps (Swann-Morton, England). Removal of this layer revealed the fibro-adipose structure superficial to the glandular tissue. This fibro-adipose tissue three-dimensional cobweb consisted of numerous small fibrous pockets, each containing adipose tissue (Figure 10a). Using twin curved forceps (Swann-Morton, England), the membrane of the adipose tissue, within each fibrous pocket, was broken and the adipose tissue was intricately detached from the fibrous wall of each pocket and carefully removed. This procedure was repeated on all of the fibro-adipose pockets of the breast to reveal the entire fibrous structure minus the adipose tissue (Figure 10b). Each pocket was then pinned with a dressmakers pin so they could be accurately counted by the chief investigator (KG). The tissue from each pocket was immediately placed into individual weigh boats for quantitative investigation.

Within each breast, the adipose tissue contents of a minimum of 20 fibrous pockets per quadrant (80 pockets per breast) were placed in weigh boats of known weights to the accuracy of three decimal places. The adipose tissue from each pocket was weighed within the first two hours after it was dissected as pilot testing found this to be the optimum duration to weigh the tissue post-dissection to prevent tissue drying.

Once all of the adipose tissue was completely dissected from the fibrous pockets, the pockets were measured using callipers (Mitutoyo, Japan) to quantify the surface area. The three-dimensional fibrous cobweb was described qualitatively and photographed within the first two hours after it was dissected (Nikon 600D, 200mm).

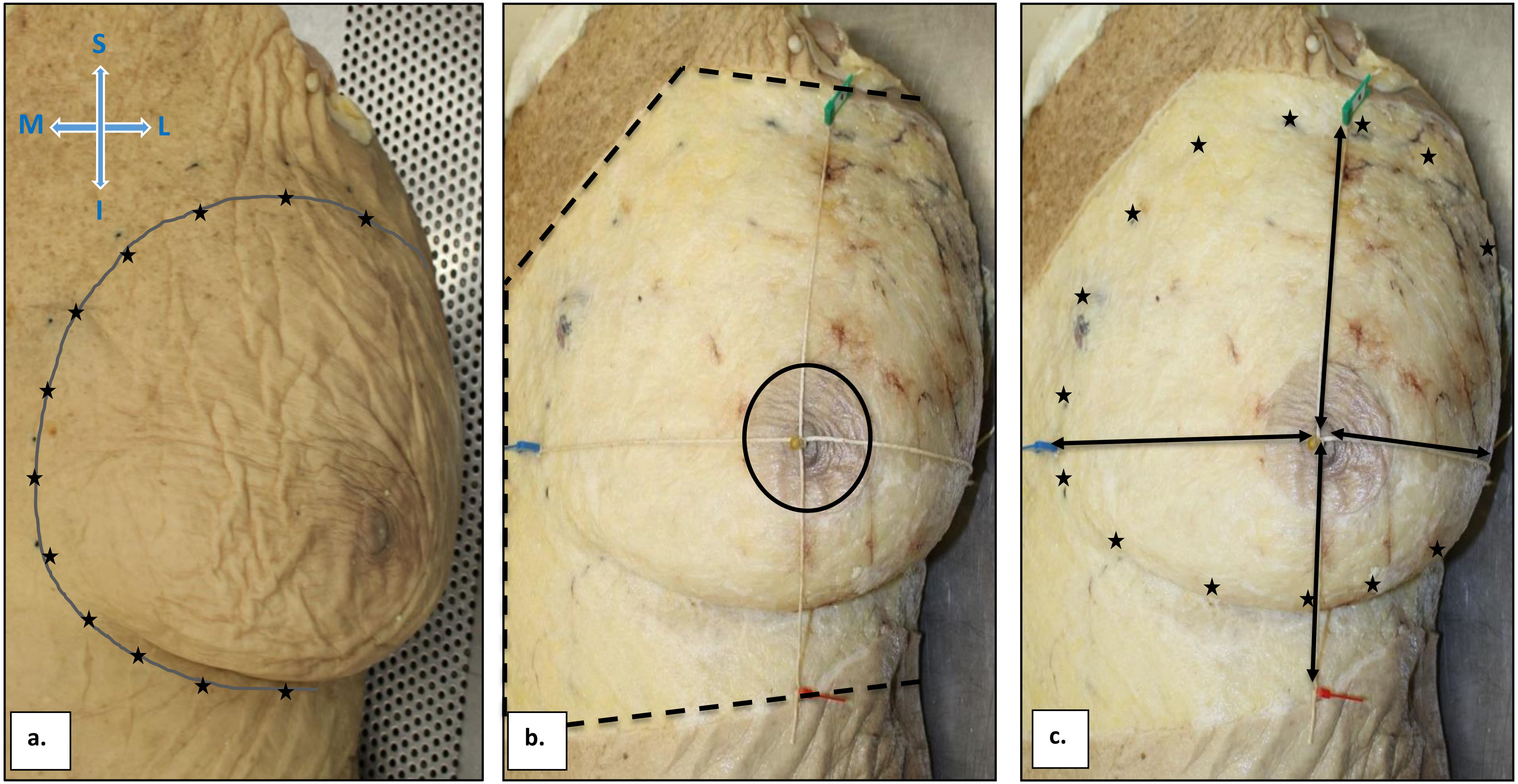

Figure 9: Coronal breast with a) Boarder outlined with permanent marker and the viscous dye injection sites (★), b) Skin removed and nipple and areola left intact, c) Skin removed, viscous dye injection sites (★) and separation of the breast into quadrants with flag pins and string.

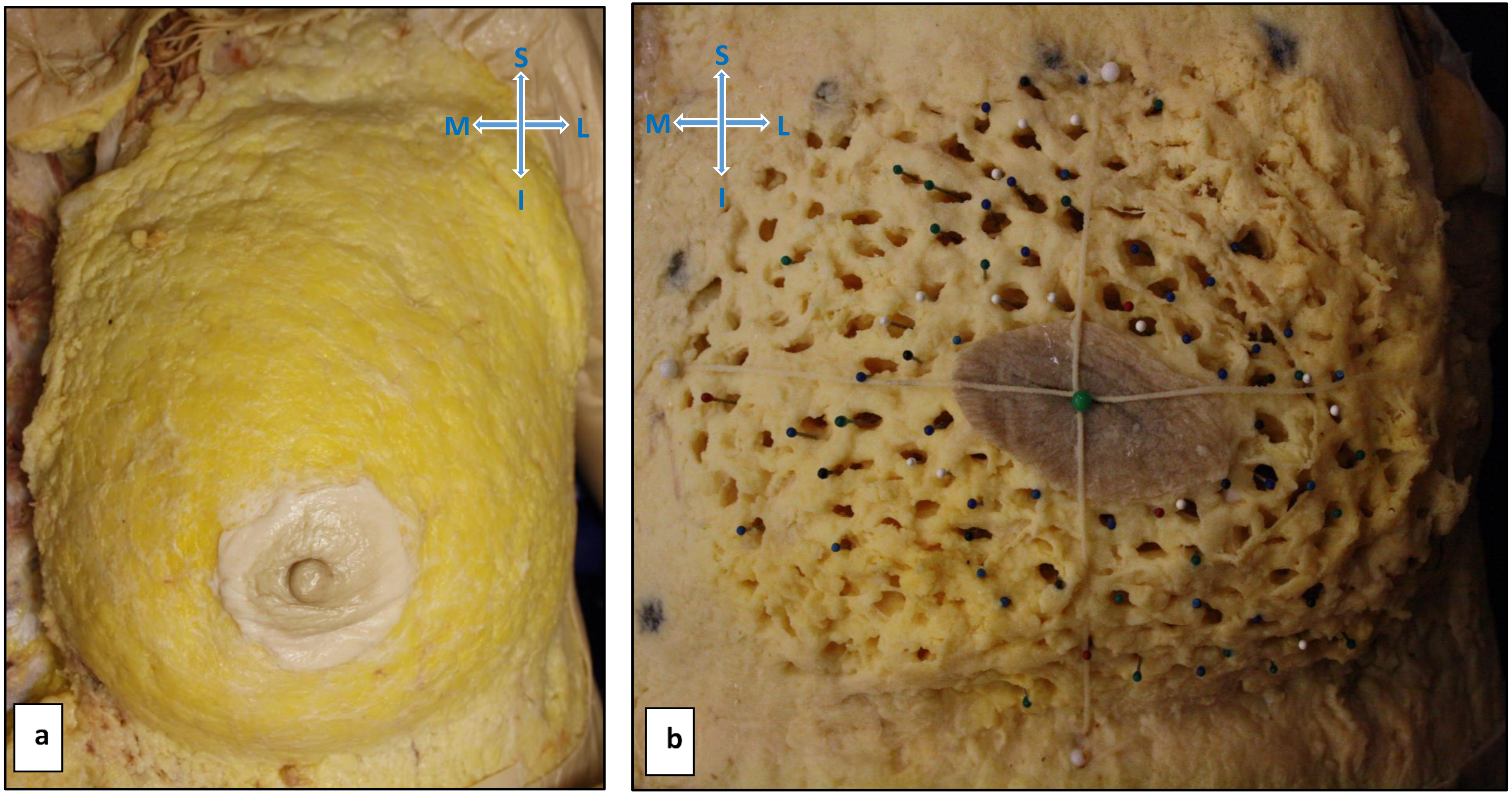

Figure 10: Coronal breast showing a) Superficial layer with adipose tissue located within fibrous pockets, b) Adipose tissue removed and pockets pinned for further quantitative analysis.

Fibro-glandular tissue

Once all of the adipose tissue was dissected from the fibrous cobweb, the remaining fibrous cobweb was then dissected from the anterior surface of the fibro-glandular tissue using sharp dissection (scalpel handle with size 10 blade and curved forceps, Swan-Morten, England) and weighed (Ohaus, Adventurer® Pro, USA). This dissection process ensured that the fibro-glandular tissue structure was not disturbed. The exposed anterior surface of the fibro-glandular was then described qualitatively and photographed (Nikon 600D, 200mm Lens; Figure 11). The fibro-glandular tissue was then removed with sharp dissection within the perimeter of the breast (scalpel handle with size 10 blade and curved forceps, Swan-Morten, England, Figure 12a). The removed fibro-glandular was then immediately weighed immediately (Ohaus, Adventurer® Pro, USA) and the structures deep to it photographed (Nikon 600D, 200mm Lens).

Fibro-adipose tissue (deep to the glandular tissue)

Dissection removal of the fibro-glandular tissue with sharp dissection (scalpel handle with size 10 blade and curved forceps, Swan-Morten, England) revealed a secondary layer of fibro-adipose tissue posterior to the fibro-glandular tissue. Removal of this deeper layer of fibro-adipose tissue within the perimeter of the breast revealed the superficial fascia of the muscles covering the anterior/lateral chest wall (Figure 12b). The dissected layer of fibro-adipose tissue lying posterior to the fibro-glandular tissue was then weighed (Ohaus, Adventurer® Pro, USA) and photographed (Nikon 600D, 200mm Lens). The aforementioned separation of the breast into four quadrants ensured that the tissue of each quadrant was kept separate from each other to allow for comparisons of the quantitative data within each region. At all levels of the coronal dissection the tissues were photographed (Nikon 600D, 200mm Lens), described and measured quantitatively.

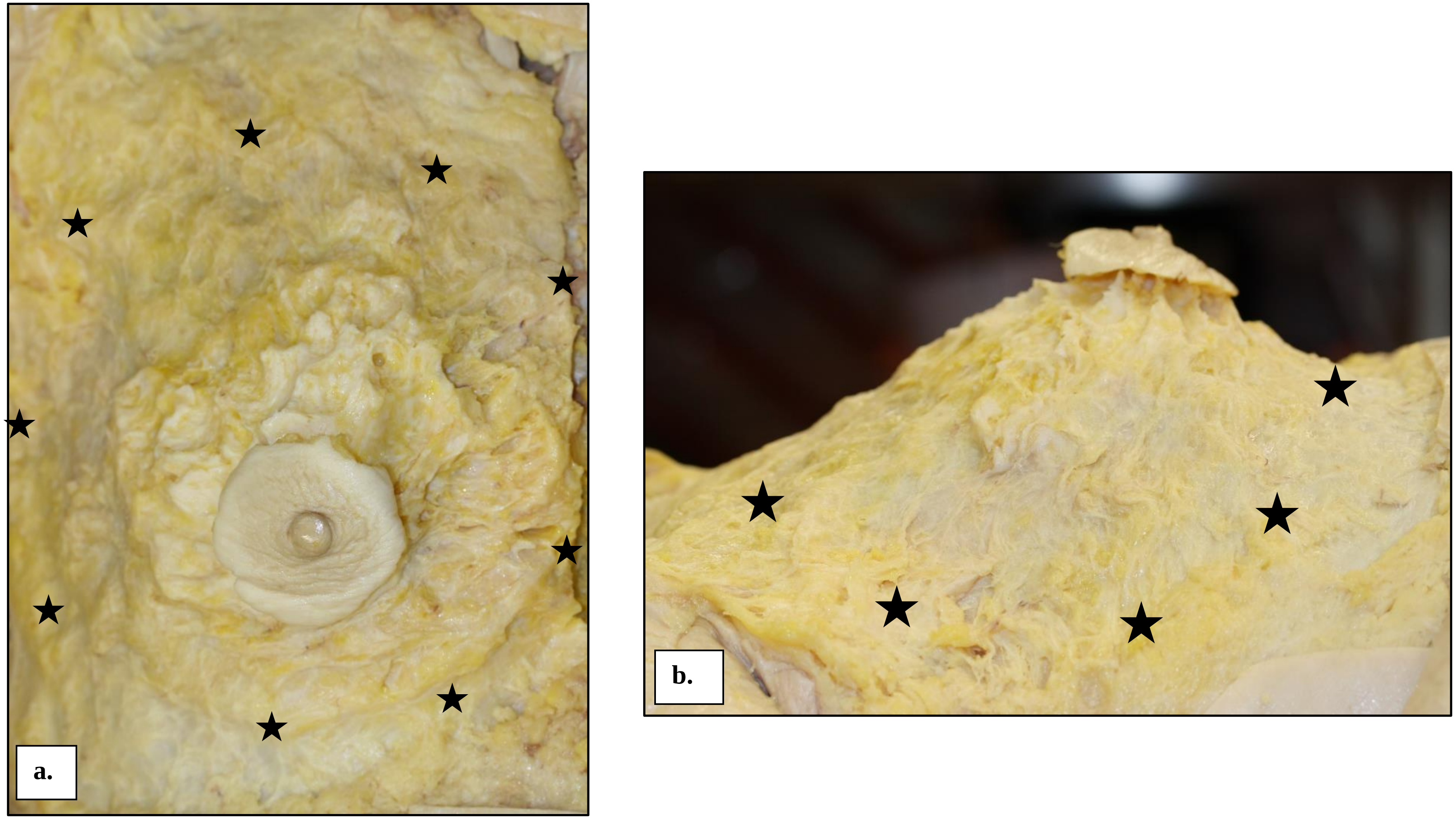

Figure 11: Fibro-glandular tissue viewed from a) Anterior aspect, b) Lateral aspect, (★) borders of the breast highlighted by viscous dye injections.

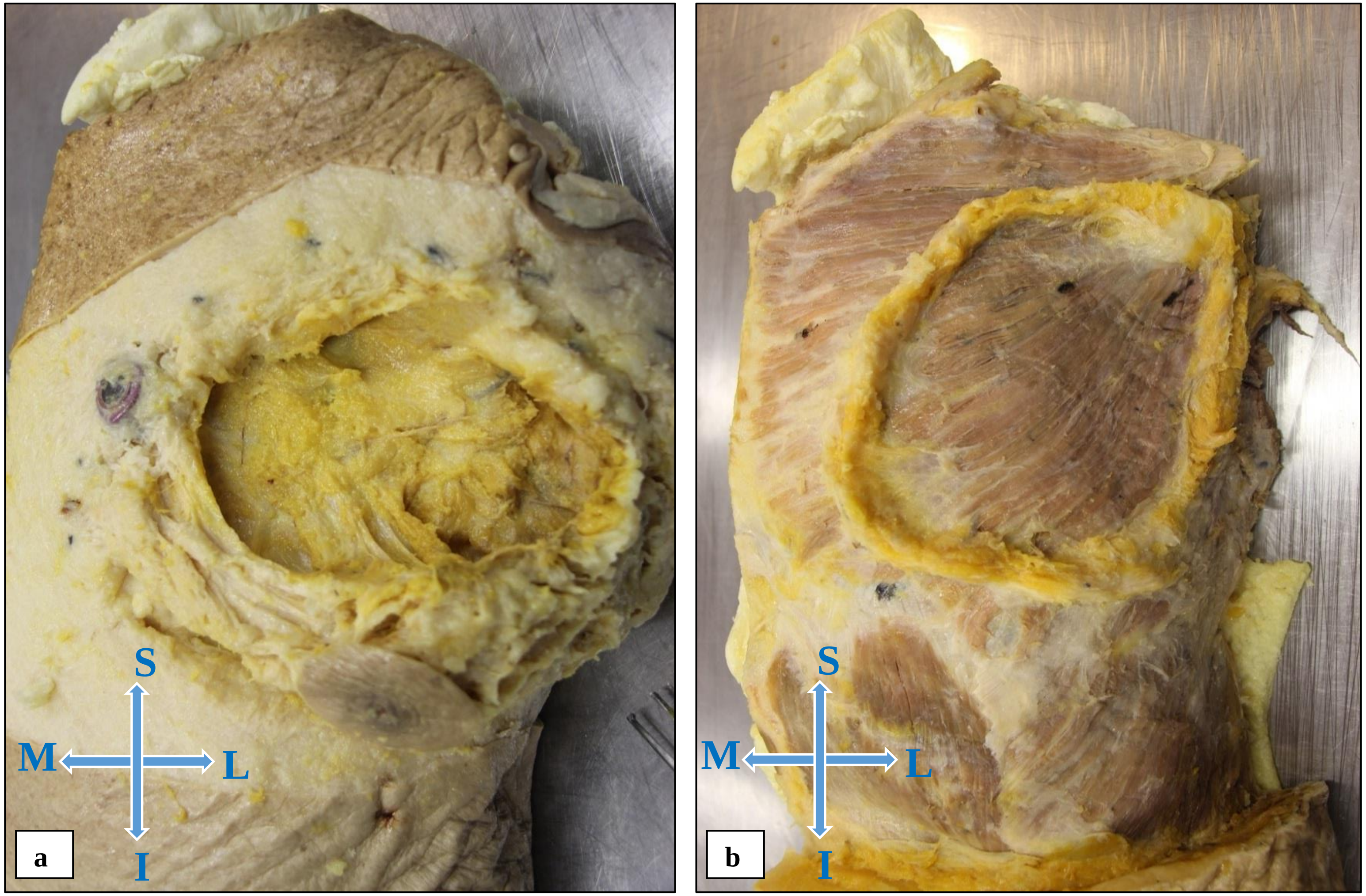

Figure 12: Coronal breast showing a) Fibro-adipose tissue deep to the fibro-glandular tissue (reflected), b) Breast removed to reveal chest wall muscles with perimeter left intact.

2.4.2 Sagittal Dissection Method

The chest plate of three female embalmed cadavers (with the skin still attached) were removed to investigate the breast in the sagittal plane following the same methods as described for the coronal dissection The boarder of the breast was marked with permanent marker (Black 70 Permanent Marker, Pelikan Artline, Australia).

Chest plate removal

The first stage of chest plate removal involved sharp dissection incisions (scalpel handle with size 10 blade and curved forceps, Swan-Morten, England) through the soft tissues of the chest (skin, subcutaneous fat and muscle). The incisions were made at the borders of the chest plate which were; (i) inferiorly - at least 2cm below the inferior boarder of the breast, (ii) laterally - posterior to the mid-axillary line and (iii) superiorly - along the superior aspect of the clavicles (Figure 13a). The incisions were then opened using spreaders (Swann-Morten, England) The remaining tissues were separated by the fingers of the chief investigator (KG) to reveal the deep bony structures, which were then cut (scalpel handle with size 10 blade and curved forceps, Swan-Morten, England) to remove the chest plate following the method described below.

On the superior aspect of the chest plate, the first rib and clavicle were exposed superior to the attachment of the subclavius muscle. Precise care was taken to gently tease the periosteum off the posterior surface of the clavicle to reflect the subclavius muscle from the posterior surface, whilst keeping the attachment to the first rib intact. The exposed clavicles were the sawed through their middle third with a bone saw (Stryker 811 Autopsy Saw, ThermoFisher Scientific, United States of America). A metal ruler was then placed under the attachment of the pectoral muscles to ensure no major blood vessel or structures in the axillary region were cut. The pectoralis major and minor muscles were then identified. The tissues in the surrounding area (brachial plexus, subclavian artery and subclavian vein) were identified and carefully separated from the posterior aspect of the pectoralis major and minor muscles. A metal ruler was placed within the newly created gap below the pectoralis major and minor muscles to protect the underlying structures (brachial plexus, subclavian artery and subclavian vein). The pectoralis major and minor muscles were then incised 3cm distal to their insertion (pectoralis major: bicipital groove of the humerus; pectoralis minor: coracoid process of the scapula) using sharp dissection (scalpel handle with size 10 blade and curved forceps, Swan-Morten, England) to reveal the ribs and intercostal muscles.

On the lateral aspect of the chest plate, the intercostal muscles were pierced through to expose ribs 1-10, which were then cut using rib-snips (Swann-Morten, England) posterior to the mid-axillary line. On the lateral aspect of the chest plate, the abdominal cavity was opened along the entire inferior boarder of the skin incision (2cm inferior to the breast) using sharp dissection (scalpel handle with size 10 blade and curved forceps, Swan-Morten, England).

Once the chest plate was detached along its superficial borders, any soft tissue attached to the deep surface of the internal surface of the chest plate (e.g. diaphragm, blood vessels, cardiac sack) were then detached from the chest plate using sharp dissection (scalpel handle with size 10 blade and curved forceps, Swan-Morten, England). The dissected chest plate was then moved away from the rest of the cadaver (Figure 13b).

Sagittal sectioning of the breast

The removed chest plate was then placed in a plastic container with the concave internal surface facing downwards. Expanding foam (Sika, Australia) was then sprayed onto the internal concave surface of the chest plate, until the concavity was filled, to form a flat surface. The expanding foam was then left to set. The set expanding foam formed a flat posterior surface which allowed a smooth transmission of the chest plate through the band saw (Mark 1, H.T. Barnes, Australia) which was necessary to ensure that the sectioning of the chest plate with the band-saw could be cut evenly. Vertical, parallel lines were than marked on the skin of the breast through the nipple and either side of the areola in the sagittal plane with a black marker (Black 70 Permanent Marker, Pelikan Artline, Australia). These landmarks were used to ensure that consistent regions of the breast were exposed amongst the three breasts dissected in the sagittal plane, which were of varying shapes and sizes.

The chest plate was then sectioned along these marked lines with a using a band saw (Mark 1, H.T. Barnes, Australia) (Figure 14a). The sawing produced four sagittal breast slices per breast, which provided six surfaces of the breast for quantitative analysis (Figure 14b). Each slice surface was photographed (Nikon 600D, 200mm Lens), described qualitatively and measured quantitatively using a microscope and its associated software (Nikon SMZ800, Australia).

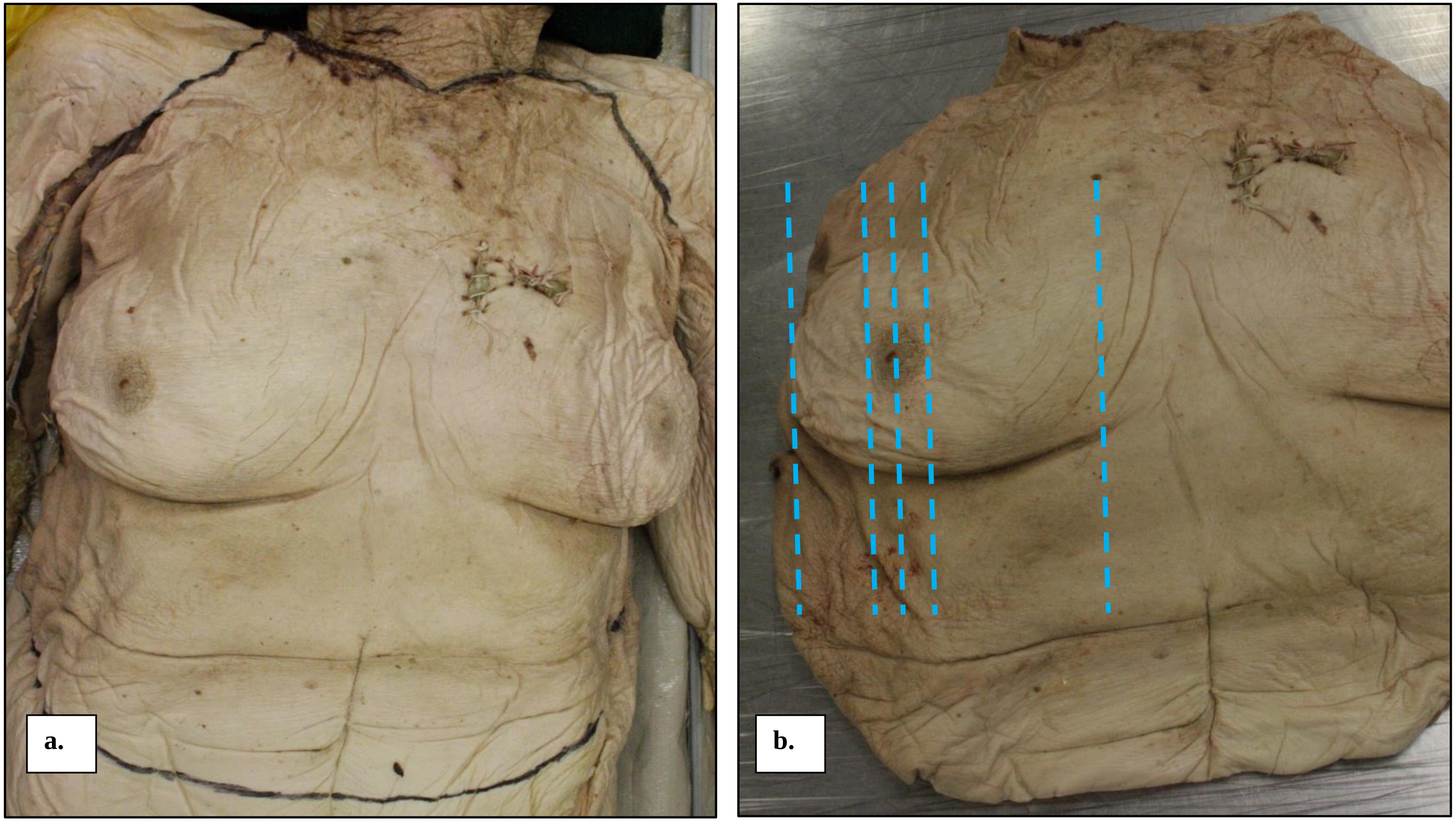

Figure 13: Sagittal dissection with a) Chest plate in-tact with chest plate removal lines marked using permanent marker, b) Chest plate removed from the cadaver with bandsaw incision lines marked.

Figure 14: Sagittal sectioning of a) Chest plate embedded in foam, which ensured a smooth and even transition through the band saw, b) Example of a mid-sagittal breast slice.

2.5 Quantitative Outcome Measures

2.5.1 Composition

2.5.1.1 Surface Area

The total surface area (cm^2) of each embalmed female breast was measured using a hand held three-dimensional white light scanner (Artec™ Eva three-dimensional Scanner, Artec Group, San Jose). With the skin removed, the breast and upper torso was scanned ensuring complete visualization of the breast tissue. The scan was then imported into Geomagic software (Geomagic Studio® software; version 12; three-dimensional Systems, South Carolina, USA) to determine total breast surface area (cm^2) and quadrant surface area (cm^2) (Figure 15a). The borders of the breast were digitally outlined following the borders previously marked with ink. The scanned breast object was removed from the scanned trunk to create a two dimensional object from which the surface area (cm^2) of the breast was calculated (Figure 15b, c). The 2D breast object was then digitally divided into four quadrants by creating two planes that intersected through the nipple (x-plane and y-plane), following the dressmaker pins previously positioned on the cadaveric breast. The surface area (cm^2) of the breast in each quadrant was then calculated.

2.5.1.2 Volume

Total breast volume (mL) and quadrant volume (mL) of each embalmed female breast was measured from the same scan of the breast used to determine surface area (cm^2). Volume required a three-dimensional object of each breast to be digitally created from the previously developed two-dimensional object of the breast. The posterior surface of the breast was created using a tangential cut plane tool to fill the anterior chest wall, once the breast was removed (Figure 15b). Based on the recommendation of previous studies [61, 86], the tangent tool was used to make a series of tangential cut planes to mimic the curvature of the anterior chest wall muscles, which the posterior surface of the breast sits on. The posterior wall of the breast object that was created was then digitally attached to the rest of the breast to create a three-dimensional model of the breast (Figure 15d). Geomagic software was then used to determine the volume (mL) of each three-dimensional breast model (n=15). The three-dimensional breast model was then digitally sectioning into four breast quadrants through the nipple as per the surface area (cm^2) measurement and the volume (mL) of each quadrant measured.

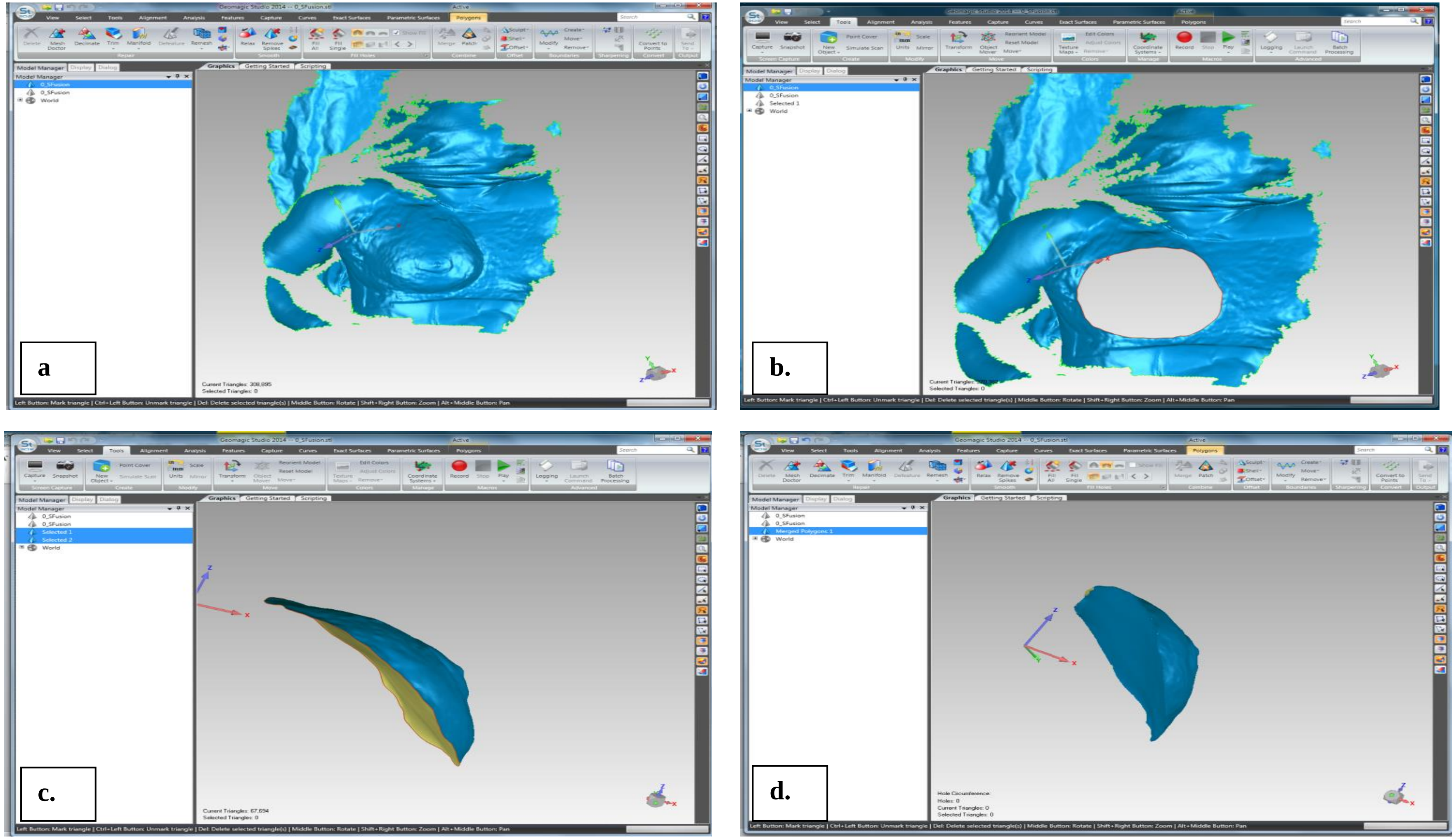

Figure 15: Geomagic analysis software, a) Full scan, b) Chest with left breast removed, c) Left breast ready for surface area calculation, d) Left breast ready for volume calculation.

2.5.1.3 Fibro-adipose mass

When investigated in the coronal plane, the total fibro-adipose tissue mass (g) was weighed (Ohaus, Adventurer® Pro, USA) in stages as it was dissected. The total fibro-adipose mass (g) was the sum of the dissected parts of the fibro-adipose tissue mass (g) both superficial and deep to the fibro-glandular tissue. The dissected fibro-adipose tissue was weighed in triplicate and the average mass (g) of the tissue within in the breast and within each quadrant was recorded for data analysis. The mass (g) of each tissue was weighed within two hours of being dissected.

2.5.1.4 Fibro-glandular mass

The total fibro-glandular tissue encased between the two layers of fibro-adipose tissue was weighed (Ohaus, Adventurer® Pro, USA) within two hours of being dissected from the deep fibro-adipose layer. The tissue was weighed in triplicate and the average mass (g) of the tissue in the breast and within each quadrant was recorded for data analysis.

2.5.1.5 Breast Mass

The total mass (g) of the fibro-adipose tissue mass (g) and the fibro-glandular tissue was summated to calculate the total breast mass (g) and the mass within each quadrant (g).

2.5.1.6 Breast Composition

The fibro-adipose and fibro-glandular composition of each breast (%) and within each breast quadrant (%) was expressed in terms of percentage of total breast mass.

2.5.2 Gross Anatomical Structure

2.5.2.1 Number of fat lobule pockets

The total number of fibro-adipose pockets within each breast and within each quadrant were manually counted and photographed (Nikon 600D, 200mL Lens) by the chief investigator (KG) from the dissection performed in the coronal plane. This was performed at the stage of dissection when the adipose tissue within each pocket was dissected. At this point, each pocket was pinned and counted (Figure 10b). Of the pinned pockets counted, 80 pockets per breast (20 per quadrant) were included in further quantitative measurements of pocket surface area and adipose tissue mass within each pocket.

2.5.2.2 Adipose mass of each pocket

The adipose tissue mass (g) within each of the 80 marked fibro-adipose pockets (20 pockets per quadrant) per breast (n=15 breasts) dissected in the coronal plane was meticulously dissected and weighed by the chief investigator (KG). Immediately after the adipose tissue was removed from the pocket, it was placed into an individual weigh boat of a known weight and weighed to the nearest 0.0001g using an anatomical scale (Ohaus, Adventurer® Pro, USA). To ensure accuracy, the adipose tissue was weighted within the first two hours after it was dissected in triplicate and the scale zeroed between each measurement. The average of the triplicate measurements was then recorded for data analysis.

2.5.2.3 Surface area of each adipose pocket

The surface area (mm^2) of each the 80 marked fibro-adipose pockets (20 pockets per quadrant) per breast (n=15 breasts) in the coronal plane dissection was measured by the chief investigator (KG) immediately after the adipose tissue within the pocket was dissected. The length and breadth of each fibrous pocket was measured medio-laterally and superio-inferiorly respectively, in triplicate using Vernier callipers (Mitutoyo, Japan) to the nearest 0.2mm. The average of each measure was recorded for data analysis.

2.5.2.4 Length of the anterior extensions of the anterior lamellae

The length of a minimum of 20 anterior extensions of the anterior lamellae per slice surface (6 breast slice surfaces per breast; n=3 breasts; therefore 120 extensions per breast) were measured by the chief investigator (KG) using an anatomical microscope (Nikon SMZ800, Australia) and its associated software. The software allowed for two set points to be created by the chief investigator (KG), one at the deep end of the Anterior Extension (Anterior Lamellae) and one at the superficial end (dermis). The straight line distance between the two points was calculated by the software. Each measurement was taken in triplicate and the average was recorded for data analysis.

2.5.2.5 The number of pockets anterior and posterior to the gland

The total number of fibro-adipose pockets located anterior and posterior to the gland were pinned with dressmaker pins and manually counted by the chief investigator (KG) from the dissection performed in the sagittal plane (Figure 14b). The measurement was performed on six sagittal breast slice surfaces (3 slices from each of the 3 breasts). Each slice was also photographed for verification (Nikon 600D, 200mL Lens).

2.5.2.6 The cross sectional surface area

The surface area (mm^2) of a minimum of 20 fibro-adipose pockets located anterior and posterior to the gland (Figure 14b) was measured using an anatomical microscope (Nikon SMZ800, Australia) and its associated software. The chief investigator (KG) used the software to digitally outline the perimeter of each pocket, from which the surface area (mm^2) was measured. All measurements were taken in triplicate and the average was recorded for data analysis.

2.5.3 Attachment of the breast to the chest wall

2.5.3.1 Muscles within the perimeter

At the last stage of the coronal investigation, the entire structure of the breast was removed within its perimeter, exposing the anterior-lateral chest wall muscles located within the perimeter of the breast (n=12 breasts). Each cadaver was photographed at this stage (Nikon 600D, 200mm Lens) and the chief investigator (KG) used software

(ImageJ, Wayne Rasband, Maryland, USA) to quantify the percentage surface area that each muscle contributed to the total surface area within the perimeter of each breast. The percentage contribution of each muscle to the total surface area per breast was recorded.

2.5.3.2 Perimeter

The perimeter of each breast dissected in the coronal plane (n=12) was measured by the chief investigator (KG) using surgical silk (Dynek, SA, Australia). The surgical silk was laid precisely around the internal boarder of the breast border and the point at which the silk reconnected with the start was noted. The length of the silk was measured in triplicate using a measuring tape in (mm) and the average was recorded for data analysis.

2.6 Qualitative Measures

Anatomical descriptions were used to describe the composition, gross anatomical structure and the attachment of the breast to the chest wall in both the coronal and sagittal planes.

2.7 Experimental Standardisation and Reliability

A number of different techniques were employed to ensure standardisation and reliability during the entire data collection phase.

2.7.1 Chief Investigator

All dissection work was carried out by the chief investigator Kathryn Gaskin (KG). This reduced inter-observational discrepancies and ensured consistency throughout the dissection process. KG has been employed by the University of Wollongong as an Anatomy Technical Officer, with four years anatomical dissection experience. As such, she has achieved a high level of competence in anatomical dissection.

2.7.2 Reliability Study

Prior to the cadaveric study, a reliability pilot study was conducted on one female cadaver aged 68, whom died of a pelvic chordoma. Each of the breasts was examined using the two different dissection methods outline above (left breast - coronal plane dissection; right breast - sagittal plane and the reliability of the various manual measurement was tested. These were the weighing of tissue samples (g), the measurement of straight-line distances with callipers (mm) and the counting of fat lobule pockets (n). Interclass correlation (ICC) were interpreted as per Cicchetti (1992) [87] as poor (< 0.40), fair (0.40-0.59), good (0.60-0.74) and excellent (0.75-1.00).

The reliability of tissue weighed using the anatomical scales (OHAUS Adventurer, Anatomical Scales, USA; see sections: 2.5.1.3, 2.5.1.4, 2.5.1.5 and 2.5.2.2 for outcome measures) was determined by weighing three small weights of known values on three separate occasions. The interclass correlation (ICC) measured across the three separate occasions was found to be 0.99, a high reliability. The reliability of tissue weighing at varying intervals subsequent to the removal from the body was conducted on ten small samples of adipose tissue and fibrous tissue from various regions of the breast. This was conducted to determine the likelihood of weight change due to drying out due to exposure with air. Weighing of the samples was conducted one hour, two hours and three hours subsequent to the dissection of the tissues. The interclass correlation (ICC) from the first hour to the third hour was found to be 0.58, a moderate reliability, and as such, tissue samples were weighed within the first two hours after dissection. The reliability of the straight line distances measured using the Vernier callipers (Mitutoyo, Japan; see sections: 2.5.2.3, 2.5.2.4 and 2.5.2.6 for outcome measures), were measured on ten tissue pockets on three separate occasions and were found to have ICC of 0.97, a high reliability. The number of pockets within the breast were manually counted (see sections: 2.5.2.1 and 2.5.2.5 for outcome measures) by KG on three separate occasions and KG was found to be highly reliable with an ICC of 1.00.

2.7.3 Cadaver Preparation and Storage

All cadavers used in the study were acquired through the University of Wollongong Body Donation Programme. All cadavers were screened for Hep B, Hep C and HIV upon arrival to the facility with positive cases rejected. All cadavers were embalmed through the left common carotid artery with at least 20L of Arterial Embalming Fluid (Genelyn, Australia) and left in the Anatomy Laboratory cool room for six months so all tissues could be perfused.

When not in use all cadavers were stored inside a blue body bag, on stainless steel body trays, in the University of Wollongong Anatomy Laboratory cool room. The temperature of the cool room is a consistent 3.5°C. Every two hours, on the even hour, between 8am and 6pm the cool room undergoes a 10-minute purge cycle to expel any of the excess formaldehyde from the room.

2.7.4 Room Conditions During Measurement

The Anatomy Laboratory at the University of Wollongong has an independent air flow system that operates solely within the laboratory space. The air is recycled 11 times every hour. There is a negative pressure gradient, which pulls all of the air from the teaching space in to the preparation space, where all investigation was conducted. The air is then moved through vents and realised into the atmosphere above the three (3) story building. The air temperature is set at 19°C. All tissue samples (fibrous tissue, adipose tissue and fibro-glandular tissue) were weighed directly after removal from the cadaver to ensure accuracy and reduce the likelihood of drying out due to the independent air flow system.

2.8 Normalising Data (absolute and relative)

During to the variation in the breast size and shape of the 15 breasts used in the study, the quantitative data of number and surface area of the fibrous breast pockets, weight of adipose tissue within each pocket, breast perimeter, and breast composition were normalized to the breast volume and surface area of each breast.

2.9 Statistical Design

The statistical analysis software used in this study was Statistix ver. 10 (Tallahassee, USA). Descriptive statistics was performed on all quantitative data. A one-way analysis of variance (ANOVA) was used to compare the quantitative data in each quadrant of the breast, with Tukeys post-hock analysis. An independent T-test was used to compare the number of pockets, mean pocket mass and mean pocket surface area in the upper and lower tertiles by volume. Pearson’s correlations were used to compare breast volume, breast surface area, breast mass, number of pockets, pocket mass and pocket surface area.

Chapter 3:

Results

3.1 Composition

3.1.1 Surface Area

The mean total breast surface area of the 15 breasts (with the skin removed) measured from the three-dimensional scans was 302±91 cm^2 (range: 187-501 cm^2; Table 5). The surface area per quadrant measured in cm^2 and as a percentage of the total surface area is displayed in Table 6 and Figure 16.The surface area was not evenly distributed amongst the breast quadrants with the cadaver in the supine position. The superior-medial quadrant had a significantly larger mean surface area than the other quadrants ($p<0.01$; mean surface area 33 %, 99.3±3 5cm^2). The superior-lateral quadrant accounted for a mean of 26 % of the total breast surface area (mean surface area 79.6±25 cm^2), which was significantly greater than the mean surface area of the inferior-medial quadrant (21 %; mean surface area 63.3±24 cm^2) and the inferior-lateral quadrant (20 %; mean surface area 60.2±26 cm^2). The mean surface areas of the inferior-medial and inferior-lateral quadrants were not significantly different to each other.

3.1.2 Volume

The mean total breast volume of the 15 breasts (with the skin removed) measured from the three-dimensional scans was 381±272 mL (range: 56-959 mL; Table 5). The volume per quadrant measured in mL and as a percentage of the total volume is displayed in Table 6 and Figure 17. As with the breast surface area, the breast volume was also not evenly distributed amongst the quadrants. The superior-medial quadrant had the greatest quadrant volume ($P<0.01$) accounting for a mean 32 % of the total breast volume (mean volume 115±90 mL). Contrary to the quadrant surface area pattern however, the quadrant volume distribution was not different amongst the other three quadrants. The superior-lateral quadrant accounted for 24 % of the volume (mean volume 101±78 mL), the inferior-lateral quadrant 23 % of the volume (mean volume 84±63 mL) and the inferior-medial quadrant 21 % of the total volume (mean volume 81±58 mL).

Table 5: Mean (SD) total breast surface area, volume and number of pockets per prosected breast.

Coronal Breast Number	Total Surface Area (cm^2)	Total Volume (mL)	Number of Pockets (n)
1	187.08	169.98	108
2	416.30	649.03	220
3	326.80	392.08	211
4	293.43	253.32	205
5	292.03	301.76	196
6	500.69	958.70	306
7	416.85	746.31	301
8	219.27	74.945	207
9	221.13	56.071	182
10	202.27	97.269	147
11	195.07	101.84	146
12	303.07	515.43	166
13	355.95	555.22	162
14	345.09	513.54	223
15	260.45	327.84	208
Mean ± SD	**302±91 cm^2**	**381±272 mL**	**199±53**

Table 6: Mean (SD) for the surface area, volume and number of pockets per quadrant.

	Superior-Medial Quadrant **Mean (SD)**	Superior-Lateral Quadrant **Mean (SD)**	Inferior-Medial Quadrant **Mean (SD)**	Inferior-Lateral Quadrant **Mean (SD)**
Surface Area (cm^2)	99.29 (35)	79.55 (25)	63.34 (24)	60.18 (26)
Volume (mL)	114.87 (89)	100.7 (78)	80.87 (58)	84 (63)
Number of Pockets (n)	61 (15)	57 (16)	43 (16)	38 (14)

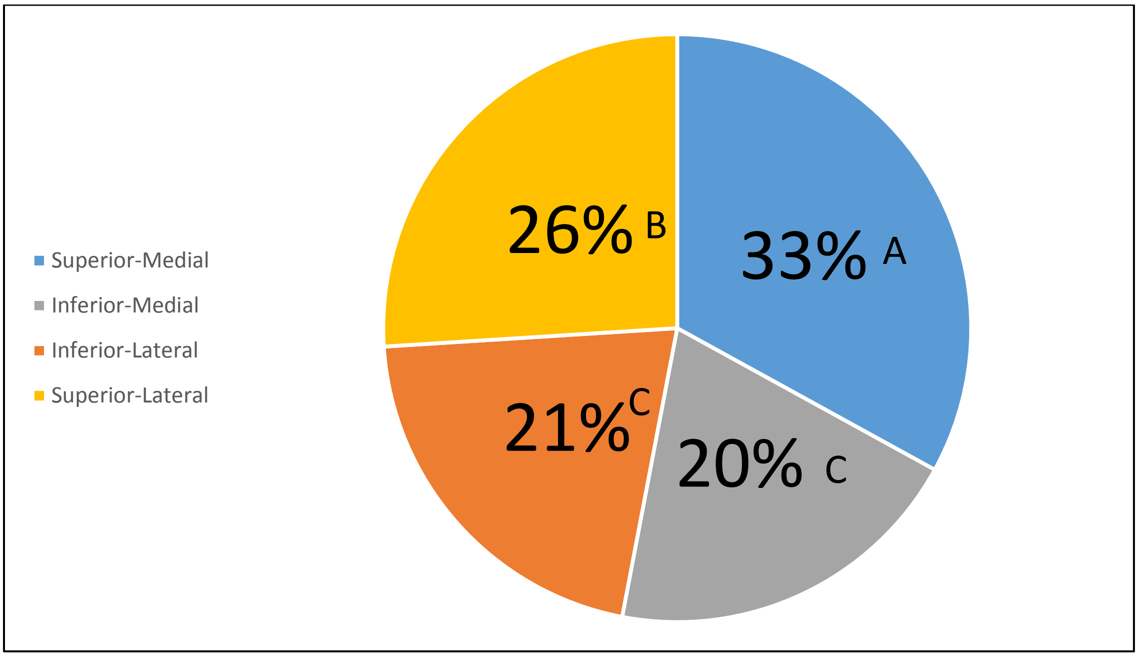

Figure 16: Mean surface area per quadrant (n=15 breasts).

[Statistical differences determined by different letters (A, B, C). The quadrants with the same letter are not statistically different and the quadrants with different letters are statistically different P<0.01]

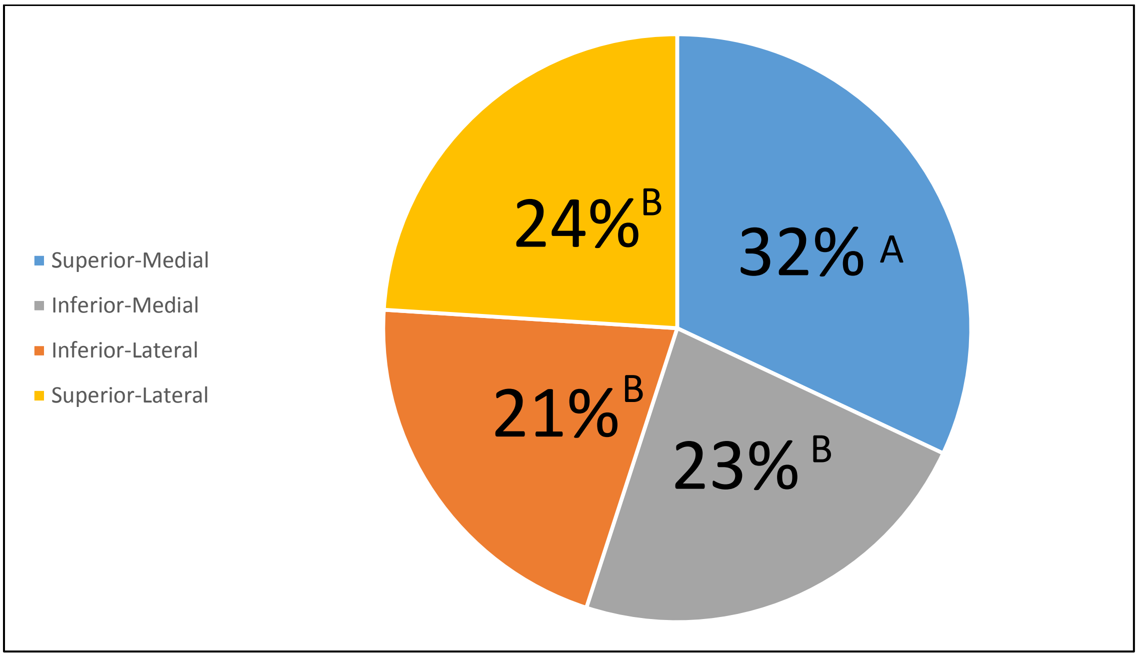

Figure 17: Mean volume per quadrant (n=15 breasts).

[Statistical differences determined by different letters (A, B). The quadrants with the same letter are not statistically different and the quadrants with different letters are statistically different P<0.01]

3.1.3 Fibro-adipose mass

The mean total fibro-adipose mass per breast of the 14 breasts dissected in the coronal plane was 172±103 g (range: 50–385 g). The fibro-adipose mass per quadrant (g) and as a percentage of the total fibro-adipose mass of the breast within each quadrant is displayed in Table 7 and Figure 18. The fibro-adipose mass within each quadrant was not evenly distributed amongst the quadrant. The superior-medial and superior-lateral quadrants were found to have a significantly greater ($P<0.01$) mean fibro-adipose mass (32 %; mean 50.8±29 g) and (30 %; mean 50.8±29 g) respectively compared to the inferior-medial and inferior-lateral quadrants (20 %; 35.3±25 g and 18 %; 29.3±19 g respectively). The fibro-adipose mass within the superior-medial and superior-lateral quadrants were not significantly different to each other and the fibro-adipose mass within inferior-medial and the inferior-lateral quadrants were not significantly different to each other (Figure 18; $P>0.05$)

3.1.4 Fibro-glandular mass

The mean total fibro-glandular mass of 14 breasts dissected and measured in the coronal plane was 184±77 g (range: 56-300 g). The fibro-glandular mass per quadrant (g) and as a percentage of the total fibro- glandular mass of the breast are displayed in Table 7 and Figure 19. The distribution of the fibro-glandular mass was evenly distributed amongst the quadrants, with no significant difference in the percentage of the fibro-glandular mass in each quadrant found ($P>0.05$). The superior-medial had 22 % (40.4±24 g) of the total fibro-glandular mass, the superior-lateral 24 % (46.3±25 g), inferior-medial 26 % (46.4±22 g) and the inferior-lateral quadrant 28 % (50.9±24 g).

3.1.5 Breast Mass

The mean combined breast mass per breast of 14 breasts dissected and measured in the coronal plane was 355±157 g (range: 105–598 g). The combined breast mass of each of the breasts in grams (g) and as a percentage of total fibro-glandular and total fibro-adipose tissue within the total breast and per quadrant is displayed in Table 7 and Figure 20. With the cadavers in a supine position, there was no significant difference found in the combined breast mass found in each quadrant ($P>0.05$). The superior-lateral quadrant had the greatest mean breast mass (102.7±56 g) with 28 % of the combined breast mass, followed by the superior-medial quadrant (91.2±52 g) with 25 %, the inferior-medial quadrant (81.7±34 g) with 24 % and the inferior-lateral quadrant (80.2±31 g) with 24 %.

Table 7: Mass (percentage) of fibro-adipose tissue, fibro-glandular tissue and total breast mass.

Breast	Fibro-adipose mass (g) (% of total mass)	Fibro-glandular mass (g) (% of total mass)	Total Breast mass* (g)
1	50.181 (47)	55.62 (53)	105.8
2	100.12 (47)	114.30 (53)	214.42
3	64.02 (46)	74.95 (54)	138.97
4	160.14 (47)	177.59 (53)	337.73
5	155.18 (50)	154.57 (50)	309.76
6	385.07 (64)	213.01 (36)	598.08
7	383.83 (64)	214.21 (36)	598.03
8	114.71 (28)	299.92 (72)	414.63
9	123.92 (48)	134.33 (52)	258.25
10	111.79 (42)	152.95 (58)	264.73
11	114.63 (43)	153.96 (57)	268.59
12	229.31 (44)	294.27 (56)	523.59
13	253.95 (46)	297.33 (54)	551.28
15	157.65 (53)	139.86 (47)	297.51
Mean ± SD (%)	171.75±102.72 (48)	184.06±76.6 (52)	355.81±156.89

*Fibro-adipose+Fibro-glandular mass=Total breast mass

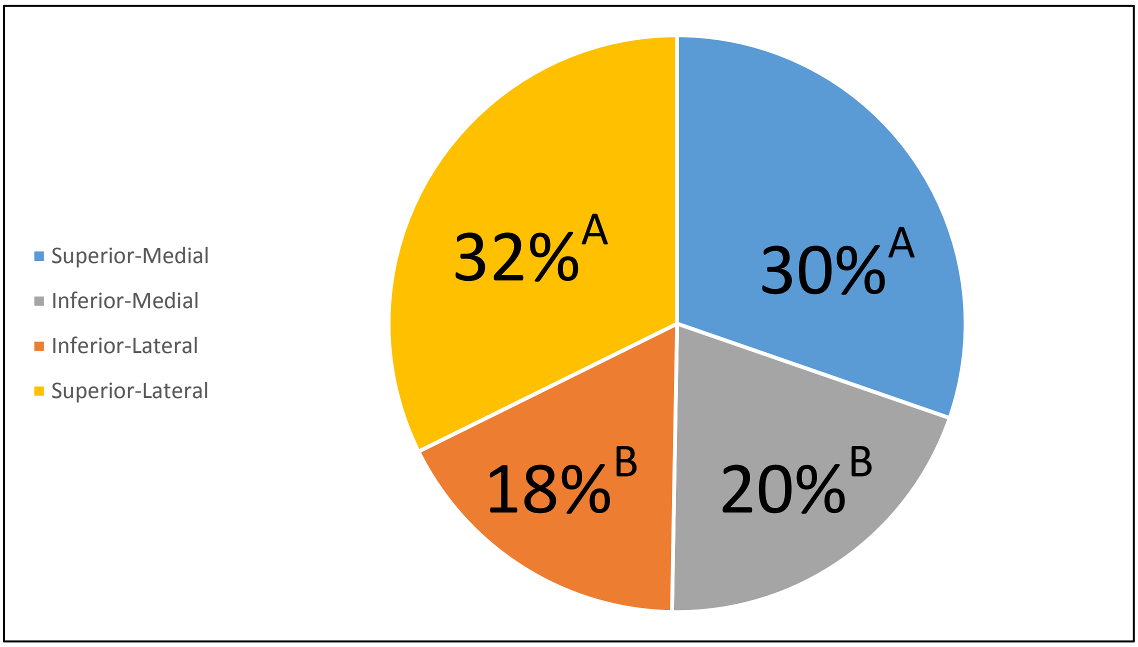

Figure 18: Mean fibro-adipose tissue mass per quadrant (n=14).

[Statistical difference determined by different letters (A, B), the quadrants with the same letter are not statistically different and quadrants with different letters are statistically different P<0.01]

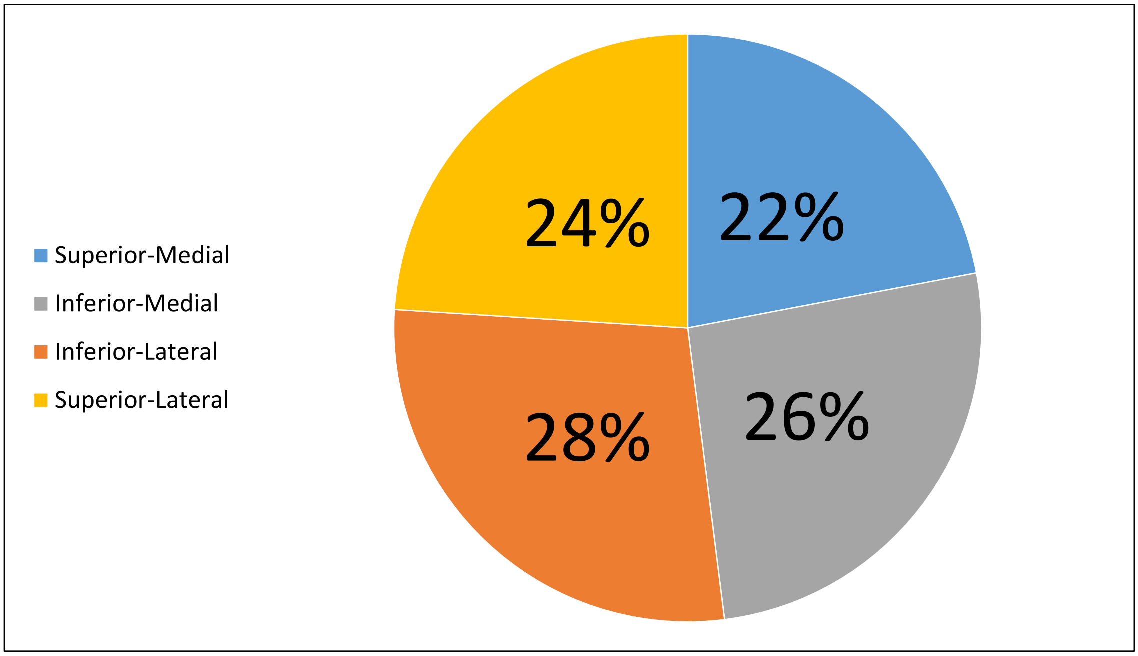

Figure 19: Mean distribution of fibro-glandular mass per quadrant (n=14).

No significant difference was found in the fibro-glandular mass in each quadrant.

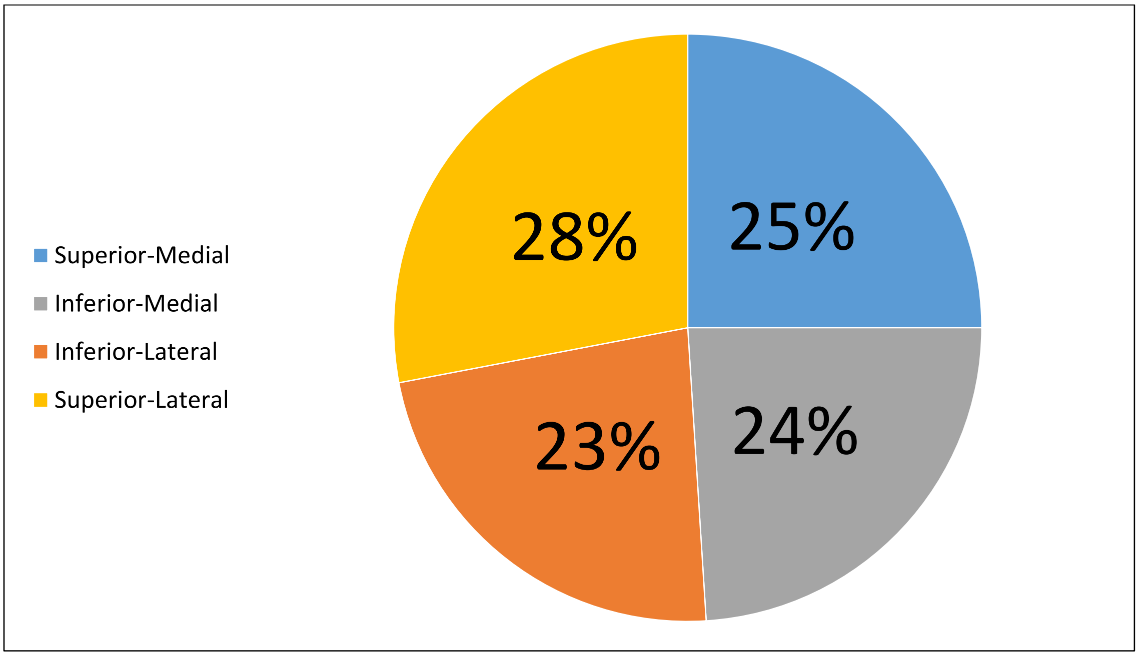

Figure 20: Mean distribution of the total breast mass (fibro-glandular and fibro-adipose mass combined) per quadrant (n=14).

No significant difference was found in the total breast mass in each quadrant.

3.1.6 Breast Composition

Breast composition by mass in terms of the percentage of fibro-glandular and fibro-adipose tissue was approximately 48 % fibro-adipose tissue (range: 45-54 %) in 11 of the 14 breasts measured (coronal plane dissection). The percentage of fibro-adipose was as low as 28 % in one breast and as high as 64 % in two of the 14 breasts dissected in the coronal plane (Table 7 and Figure 21).

The percentage composition of fibro-adipose and fibro-glandular tissue by mass within each of the four quadrants varied, with a mean of 48 % fibro-adipose tissue and 52 % fibro-glandular tissue (Table 7 and Figure 22). With the cadavers in a supine position, there was no significant difference found in the percentage composition of fibro-adipose and fibro-glandular tissue by mass in the superior-lateral quadrant (32 %) and superior-medial quadrant (30 %) or between the inferior-lateral quadrant (18 %) and inferior-medial quadrant (20 %; $P>0.05$). There was however significance differences between percentage composition of fibro-adipose and fibro-glandular tissue between the quadrants in the superior aspect of the breast relative to the quadrants in the inferior half of the breast.

3.1.7 Breast mass in relation to breast volume

Breast volume was found to increase with both total breast mass (fibro-adipose + fibro-glandular mass) and fibro-adipose mass. A moderate correlation was found between total breast mass (fibro-adipose + fibro-glandular mass) and breast volume ($r^2 = 0.5769$) and a strong positive correlation was found between fibro-adipose mass and breast volume ($r^2 = 0.\ 7758$). Fibro-glandular mass was not found to have a relationship with breast volume as a very weak correlation was found between the fibro-glandular mass and breast volume ($r^{2=}$ 0.1414; Figure 23).

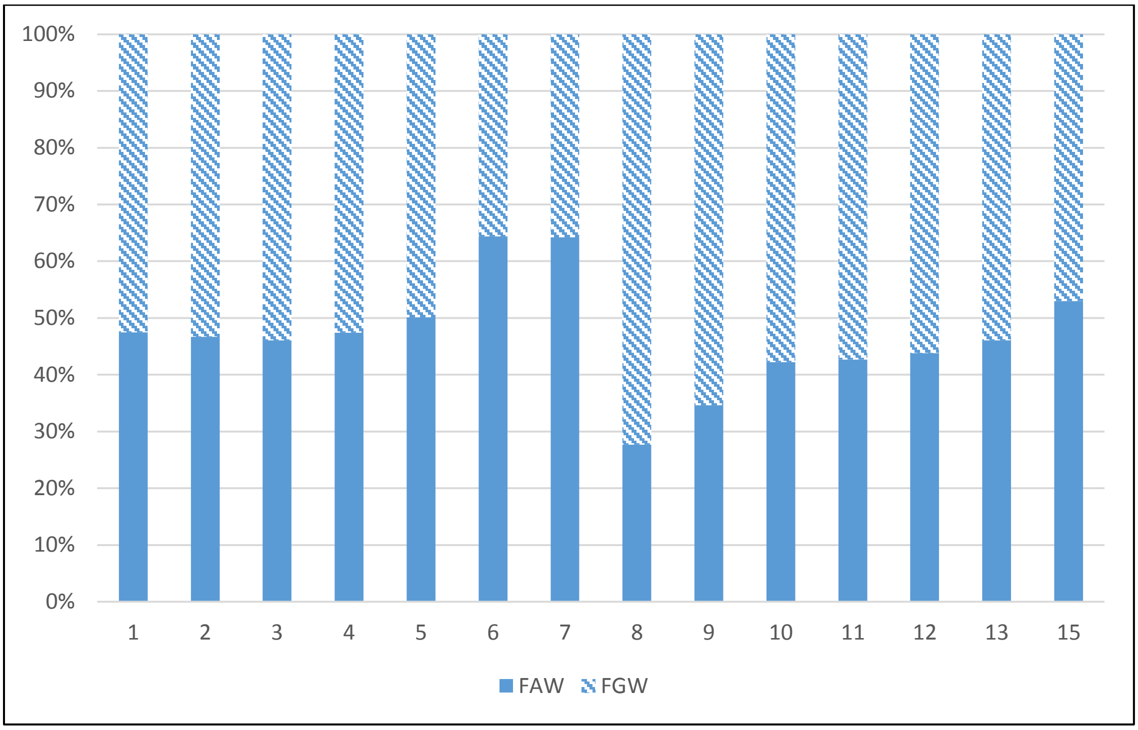

Figure 21: Breast composition as percentage of fibro-adipose and fibro-glandular mass within each breast (n=14).

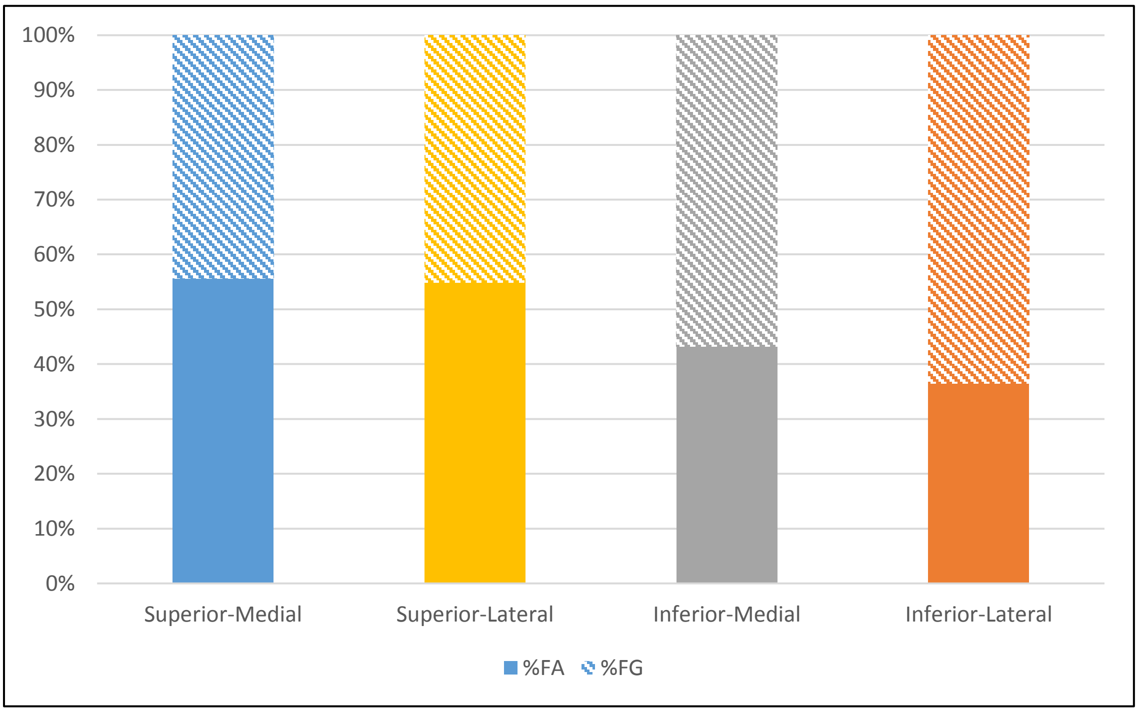

Figure 22: Mean breast composition per quadrant as percentage of fibro-adipose and fibro-glandular mass (n=14).

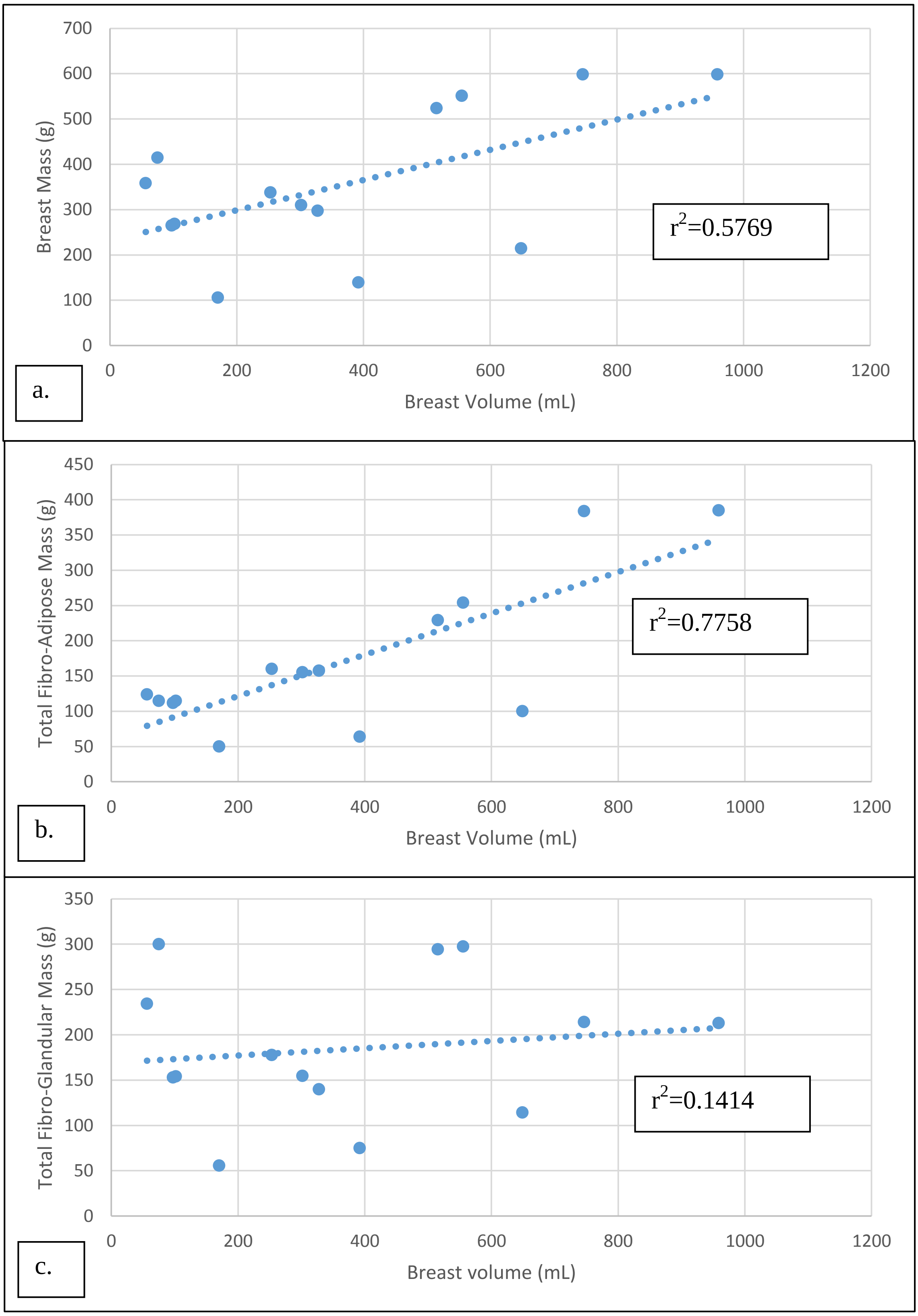

Figure 23: Correlation of a) Combined total breast mass and breast volume, b) Fibro-adipose breast mass and breast volume, and c) Fibro-glandular breast mass and breast volume (n=14; p<0.01).

3.2 Gross Anatomical Structure

3.2.1 Coronal Qualitative Measures

External breast

The external surface of the breast (skin on) formed a convex mound extending anteriorly from the chest wall and converging at the nipple in all of the 15 breasts dissected. With the cadavers in a supine position, the nipple of the breast was consistently situated slightly inferior to the mid-horizontal line and slightly lateral to the mid-vertical line of each breast. The overall shape and size of the nipple and areola varied between a circular and an oval shape. Six of the 15 breasts had circular nipples with a diameter of 2-4cm (Figure 24a), and the remaining nine had oval shaped nipples, with larger diameters 5-8cm (Figure 24b).

Subcutaneous adipose tissue

Once the skin was removed using sharp dissection, a thin layer of adipose tissue 1-2mm thick was exposed which formed a “cake icing like surface” over the superficial surface of the breast. This superficial layer of adipose tissue was very easily scraped off the external breast surface using blunt dissection and did not appear to contribute to the fibro-adipose cobweb structure lying deep to it.

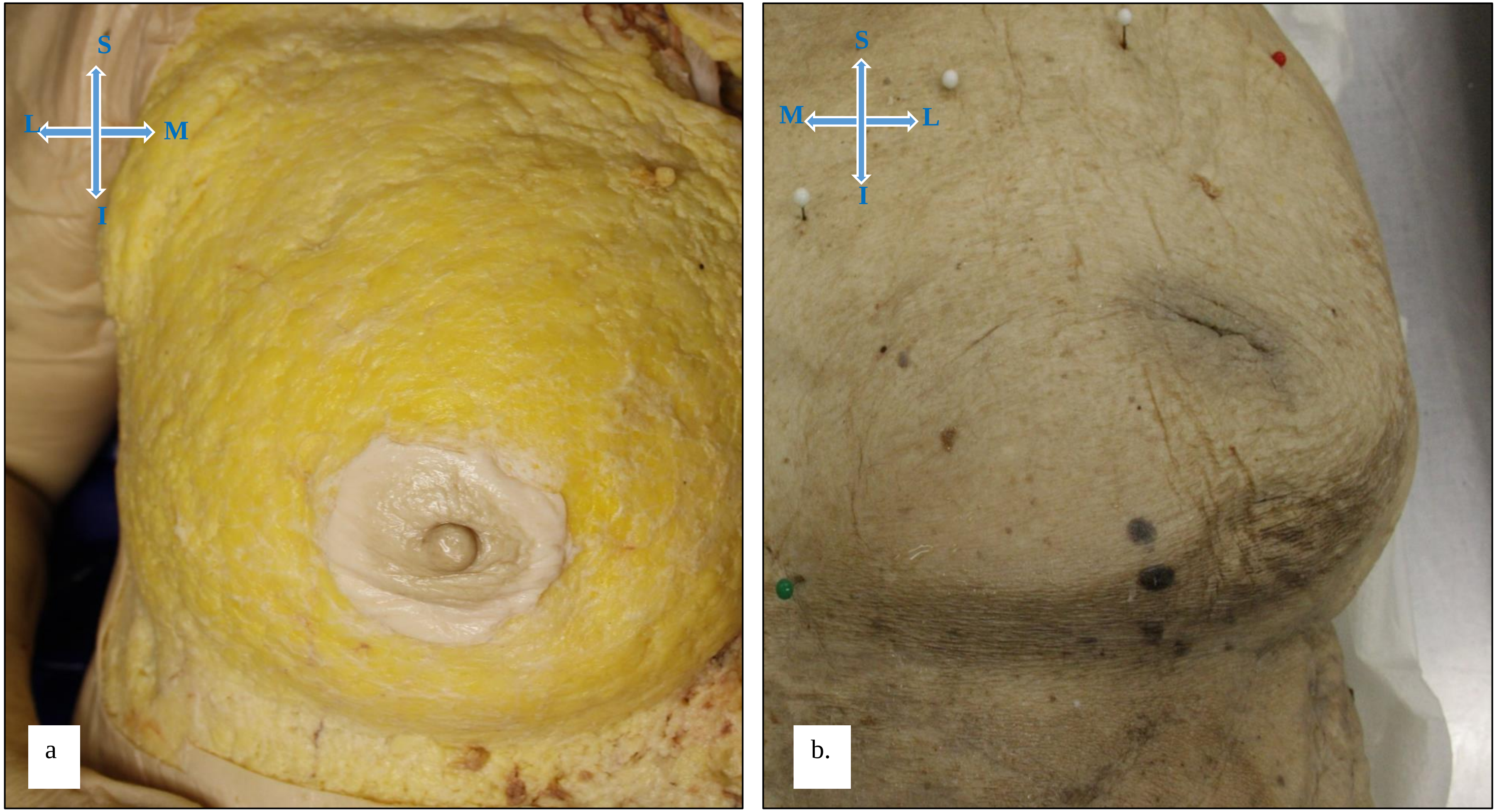

Figure 24:Coronal breast showing a) Round nipples (skin removed), b) Oval nipple (skin intact).

Fibro-adipose structure

The bulk of the external mound of the breast was formed by a three-dimensional fibro-adipose cobweb structure (Figure 25), which was located deep to the superficial surface layer of subcutaneous adipose tissue. This three-dimensional breast mound was formed by a scaffolding of fibrous tissue, which formed a vast collection of fibrous tissue pockets, with adjacent pockets sharing their walls. Each pocket was completely filled with adipose tissue, such that a fairly flat external surface was formed. In 12 of the 15 breasts, the adipose tissue was easily dissected from the fibrous walls of the pocket using blunt dissection. However, in three breasts it was very difficult to separate the adipose tissue from the fibrous pocket with blunt dissection as the fibrous tissue appeared to be thinner, which made the separation of tissues difficult without destroying tissue.

Regional variation was found in the surface area and depth of the individual pockets within the three-dimensional cobweb structure. In the superior-medial quadrant, the surface area of the pockets was the largest and in the superior-lateral quadrant, the depth of the pockets was the deepest (Figure 26). The shape of the pockets also exhibited regional variations in the majority of the breasts (13 of the 15 breasts dissected in the coronal plane). The pockets were square shaped with rounded edges in the superior two quadrants of the breast and more of an oval shape in the inferior two quadrants of the breast. Two of the 15 breasts however exhibited no regional variation in the shape of the pockets, having oval shaped pockets in all of the quadrants.

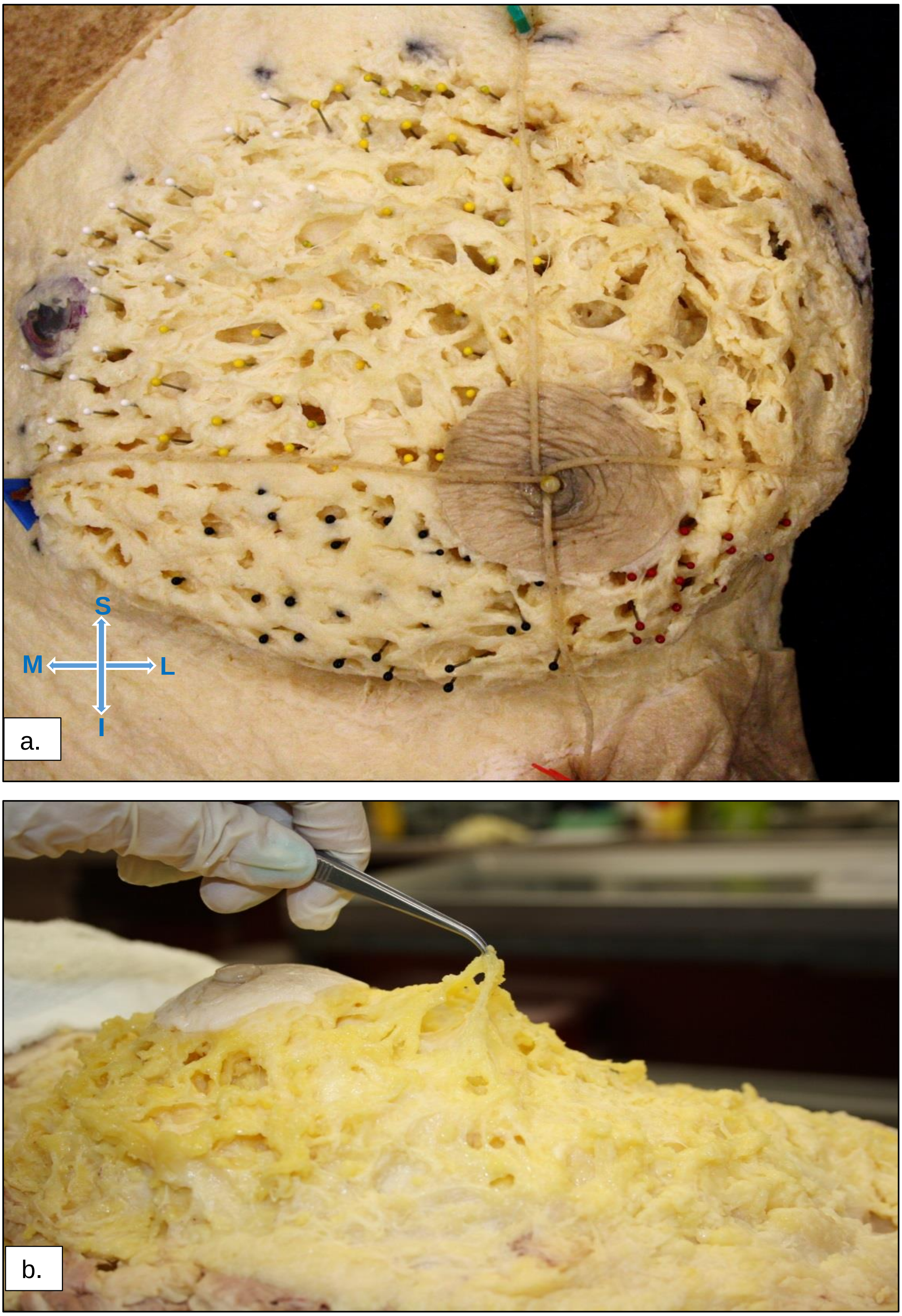

Figure 25: Fibrous cobweb of the breast from the a) Anterior aspect and b) Sagittal aspect.

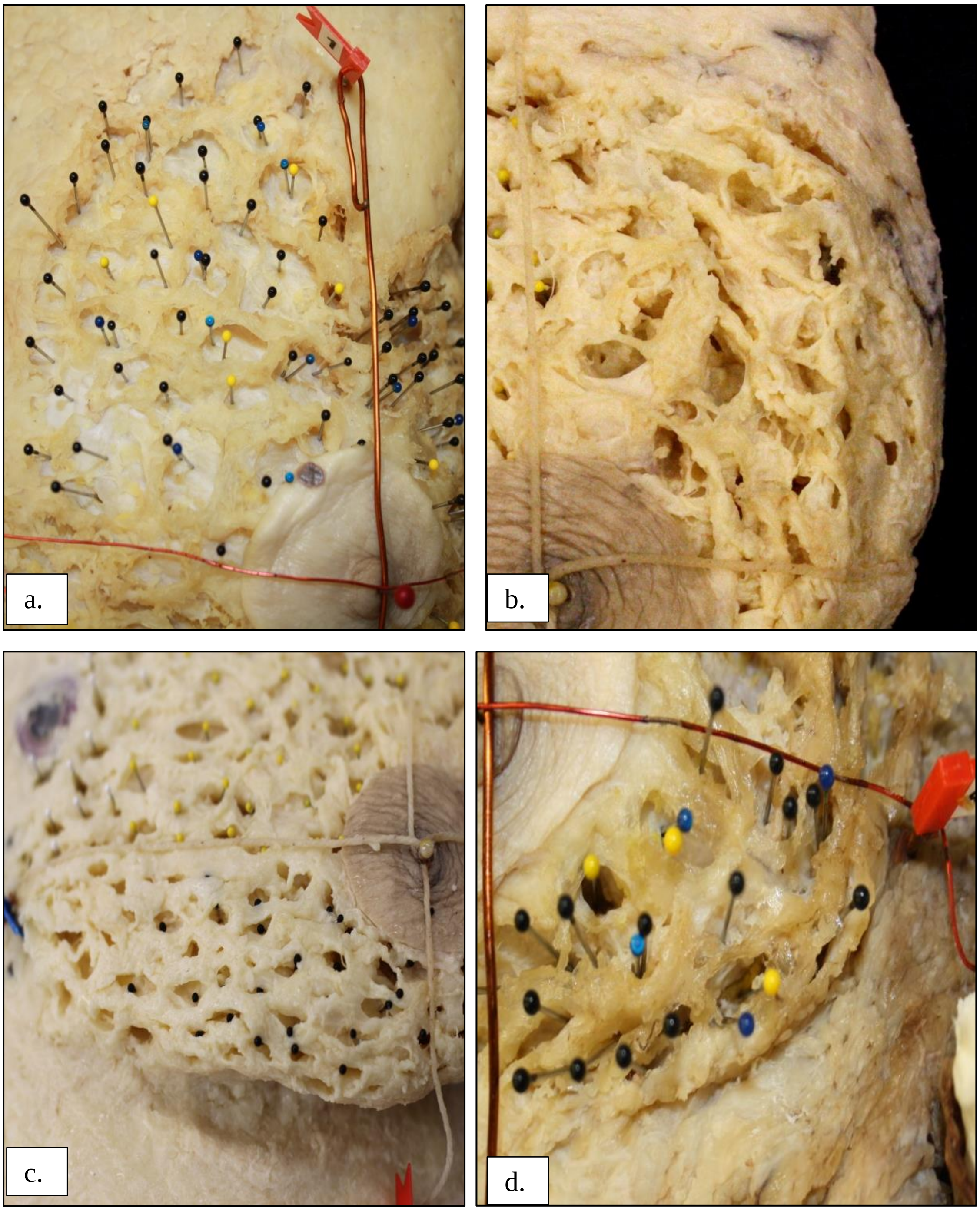

Figure 26: Example of pocket variation shape amongst the quadrants of the breast. Large square-shape pockets in the superior quadrants a) Superior-medial quadrant of small breast, b) Superior-lateral quadrant of large breast, and smaller oval shape pockets in the inferior quadrants c) Inferior-medial quadrant of a large breast, d) Inferior-lateral quadrant.

Fibro-glandular tissue

The fibro-glandular tissue was located deep to the bulk of the fibro-adipose cobweb structure and adhered to a deeper, thinner and flatter layer of fibrous-adipose tissue requiring sharp dissection to remove it. Within the non-uniform fibro-glandular structure, the glandular tissue (gland and ducts) could not be differentiated macroscopically from the fibrous tissue. In all 15 breasts, the fibro-glandular tissue was located within the central region of the breast, with the bulk of the gland located inferior to the nipple (Figure 27a). In the majority of the breasts, (12 of the 15 breasts), the fibro-glandular structure extended unevenly into the all four of the quadrants. Three of the breasts had fibro-glandular located in only three of the four quadrants, with no fibro-glandular tissue found in the superior-medial quadrant.

Fibro-adipose tissue (deep to the fibro-glandular tissue)

Deep (posterior) to the fibro-glandular tissue, the thinner and flatter layer of fibrous-adipose tissue did not have the same three-dimensional cobweb structure as the bulk of the fibrous-adipose tissue structure superficial to the fibro-glandular tissue (Figure 27b). Any pocket-like structures identified in this layer were very fragile and broke with blunt dissection. This deeper fibrous-adipose tissue layer required only blunt dissection only to separate it from superficial fascia of the following muscles, pectoralis major (sternal head), serratus anterior (anterior fibres), rectus abdominals (superior fibres) and external oblique muscles (superior fibres)

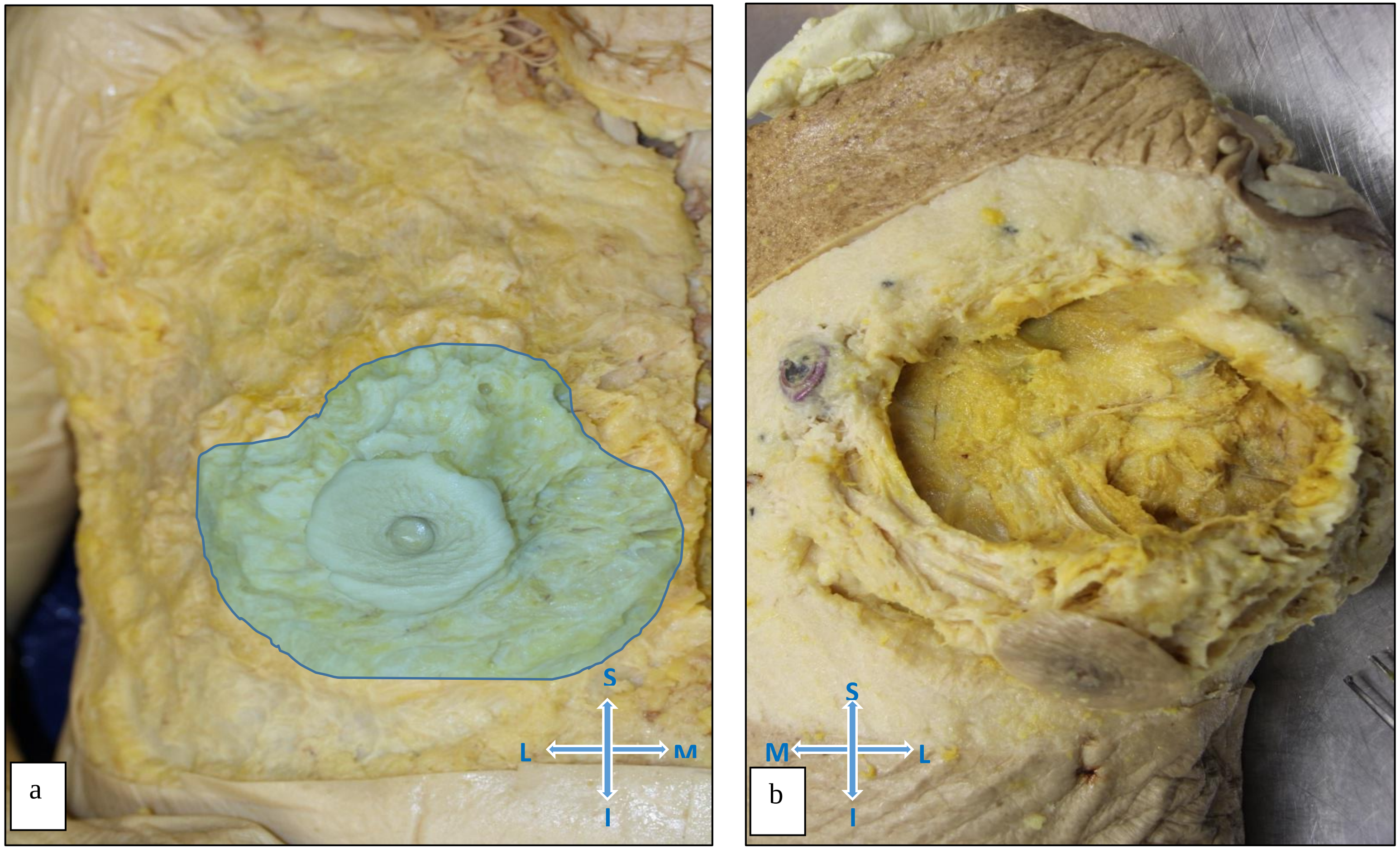

Figure 27: Coronal breast showing a) Relative size and shape of the fibro-glandular tissue, b) Fibro-glandular tissue reflected showing the deeper layer of fibro-adipose tissue situated posterior to the fibro-glandular tissue.

3.2.2 Number of fibro-adipose tissue pockets

The mean number of fibro-adipose tissue pockets found in the 15 breasts dissected in the coronal plane was 199±53 (range: 108 – 306; Table 5). The mean number of pockets per quadrant (n) and as a percentage of the total number of pockets within the breast is displayed in Table 6, Figure 28 and Figure 29. The numbers of fibro-adipose tissue pockets per quadrant were not evenly distributed and the pattern of fibro-adipose tissue pocket distribution was the same pattern as the surface area distribution per quadrant. The superior-medial quadrant had significantly more pockets than the other quadrants containing a mean of 31 % of the total number of pockets (mean number pockets 61±15) ($P<0.01$). The superior-lateral quadrants contained significantly more pockets than the inferior-medial and inferior-lateral quadrants ($P<0.01$) with 29 % of the pockets (mean number pockets 57±16). The numbers of pockets in the inferior-medial and inferior-lateral quadrants were not significantly different from each other ($P>0.05$, Figure 28). The inferior-medial quadrant contained 21 % of the pockets (mean number pockets 43±16) and the inferior-lateral quadrant contained 19 %, of the pockets (mean number pockets 38±14).

3.2.3 Number of fat lobule pockets in relation to breast volume and surface area

The number of pockets per breast was found to increase both with breast volume and surface area ($P<0.05$), with a strong positive correlation ($r^2=0.7372$) found between breast volume and number of pockets within the breast (Figure 30) and between breast surface area and the total number of pockets ($r^2=0.8064$). Although there was no significant difference in the number of pockets per breast in the largest breasts by volume (upper tertile) compared to the smallest breasts by volume (lower tertile). There was a trend however towards a higher number of pockets in the breasts of the upper tertile by volume compared to the breasts of the lower tertile breasts by volume (Table 8). A strong positive correlation was also found between total breast volume and total breast surface area ($r^{2=}$0.9200) ($p<0.01$) (Figure 30).

When the quadrants were compared on the three variables of surface area, volume and number of pockets, with the body in a supine position, the superior-medial quadrant had the greatest mean distribution of surface area (33 %), volume (31 %) and number of pockets (31 %). In contrast, the inferior-lateral quadrant had the smallest mean distribution of surface area (26 %) and number of pockets (29 %). The inferior-medial had the smallest mean distribution of volume (24 %) when compared to the remaining three quadrants (Figure 16, Figure 17 and Figure 29).

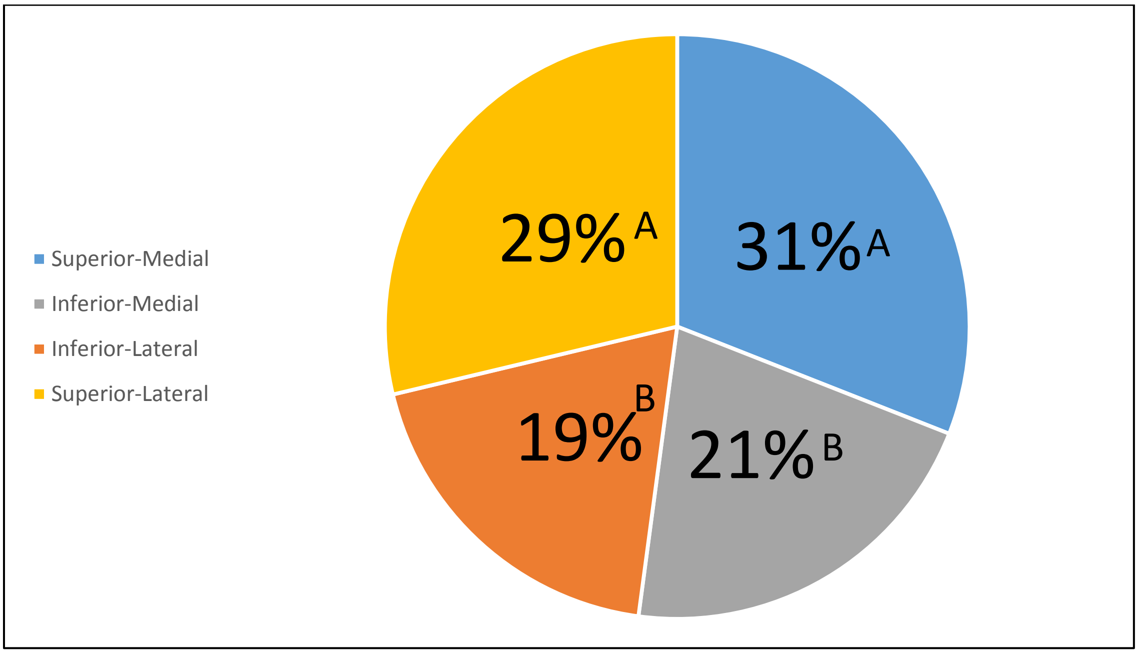

Figure 28: Mean number of pockets per quadrant (n=15 breasts).

[Statistical differences determined by different letters (A, B) whereby the quadrants with the same letter are not statistically different and the quadrants with different letters are statistically different (P<0.01]

Table 8: Mean number of pockets, pocket mass and pocket surface area in the upper and lower tertiles by volume.

	Upper Tertile by Volume	Lower Tertile by Volume	Significance
Mean Pocket Number	231.00	158.00	P>0.07
Mean Pocket Mass (g)	0.43	0.48	P>0.05
Mean Pocket Surface Area (mm^2)	72.48	86.41	P>0.05

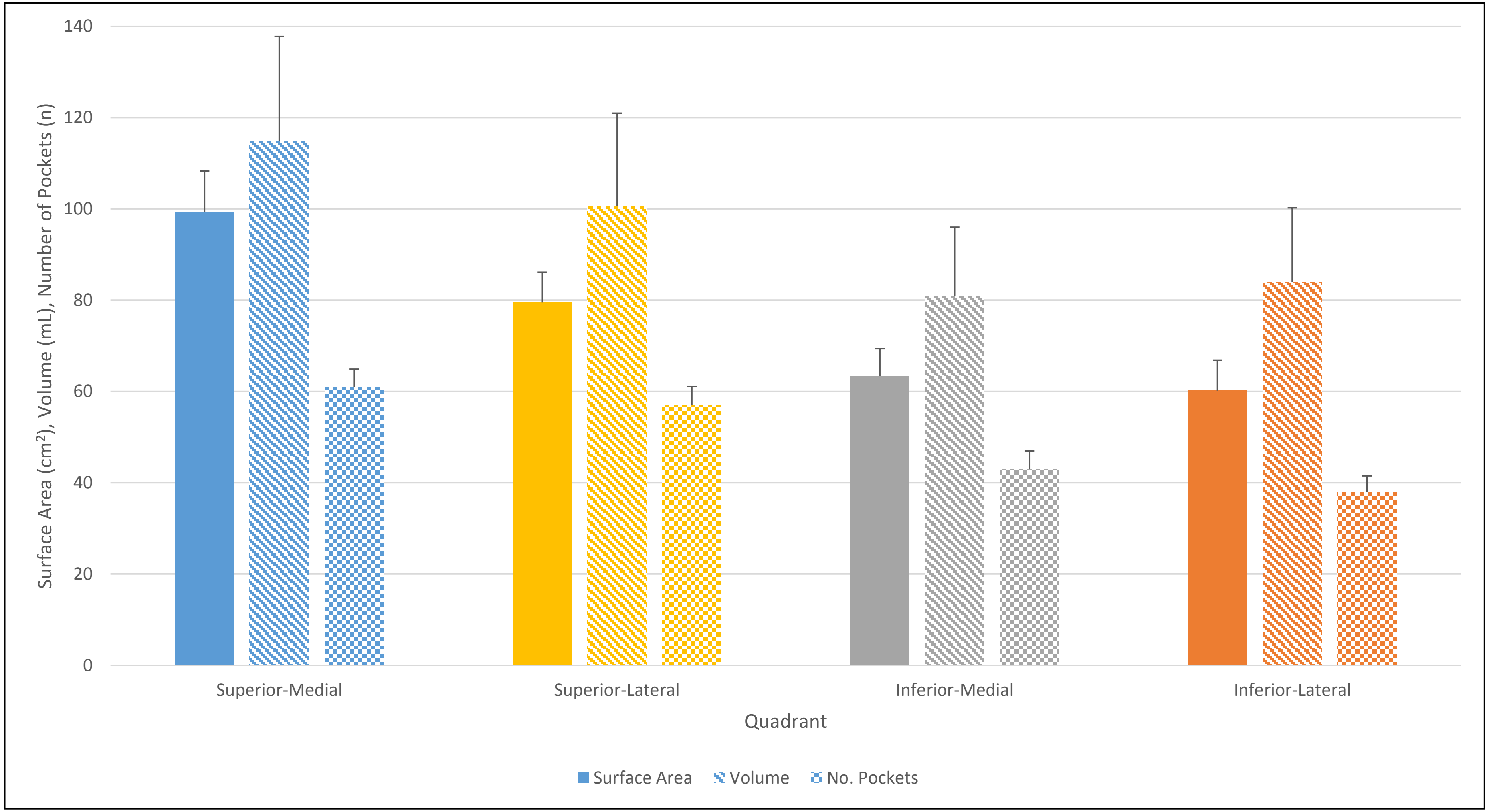

Figure 29: Mean surface area, volume and number of pockets per quadrant (Mean and SEM) in each quadrant.

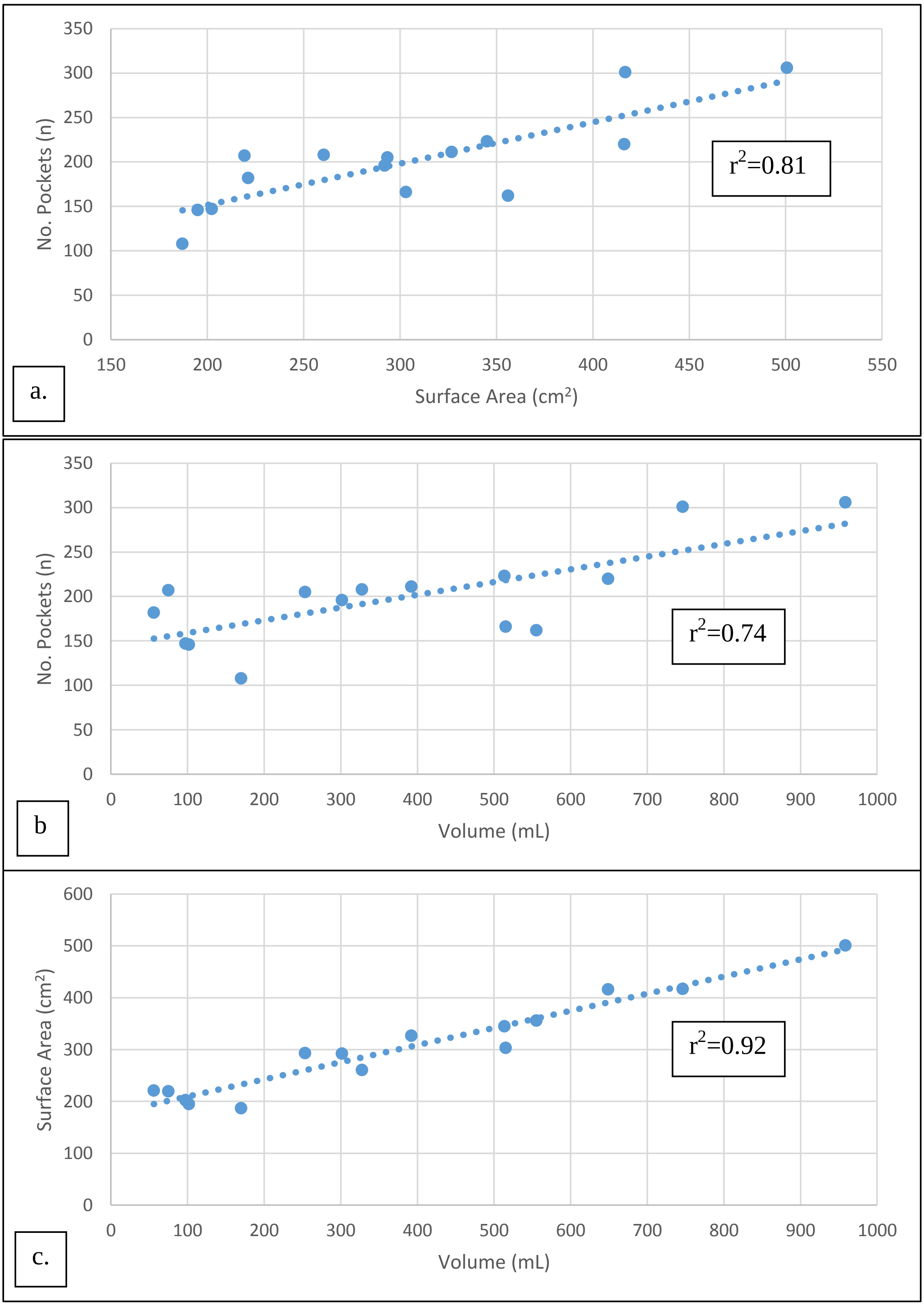

Figure 30: Correlation graphs of a) Total breast surface area to total breast number of pockets, b) Total breast volume to total breast number of pockets, and c) Total breast volume to total breast surface area per breast (n=15) (p<0.01).

3.2.4 Adipose mass of each pocket

The size of each of the adipose tissue pockets was determined from two measures, the mass of the adipose tissue encased within the pockets (g) and the surface area of the adipose tissue pockets measured immediately after the adipose tissue was dissected (cm^2). The adipose tissue mass per pocket of each of the 80 pockets per breast measured (20 pockets per quadrant per breast) of the 15 breasts dissected in the coronal plane is displayed in Figure 31. The mean adipose tissue mass per pocket of these 80 pockets measured in each of the 15 breasts displayed in Figure 32. The overall mean adipose tissue mass per pocket of the 80 pockets measured in the 15 breasts as a group was 0.4±0.15 g (range: 0.26- 0.61 g).

When isolated pocket size (mass) was compared within each quadrant, the mean pocket mass within the superior-lateral quadrant was the largest (0.49±0.19 g; Figure 33) and significantly larger ($P<0.05$) than the mean pocket mass of the other three quadrants. The mean pocket mass within the inferior-lateral quadrant was significantly smaller than the other three quadrants (0.34±0.12 g) ($P<0.05$) and the mean pocket mass in the superior-medial quadrant (0.43±0.11 g) and the inferior-medial (0.36±0.11 g) were not significantly different ($P>0.5$) from each other (Figure 34).

3.2.5 Adipose mass within each pocket in relation to breast volume and surface area

In contrast to the number of pockets per breast, which was found to have a high positive correlation with both increases in breast volume and surface area, the pocket size (measure by the adipose tissue mass within each pocket) was not found to vary with breast volume or breast surface area. A weak positive correlation was found between the mean adipose tissue mass of each pocket and total breast volume (r^2=0.0588; Figure 39) and a very weak negative correlation was found between the mean adipose tissue mass of each pocket and the total breast surface area (r^2=-0.0055). There was also no significant difference found in the adipose mass within each pocket of the largest breasts by volume (upper tertile) compared to the smallest breasts by volume (lower tertile) $P>0.05$; (Table 8).

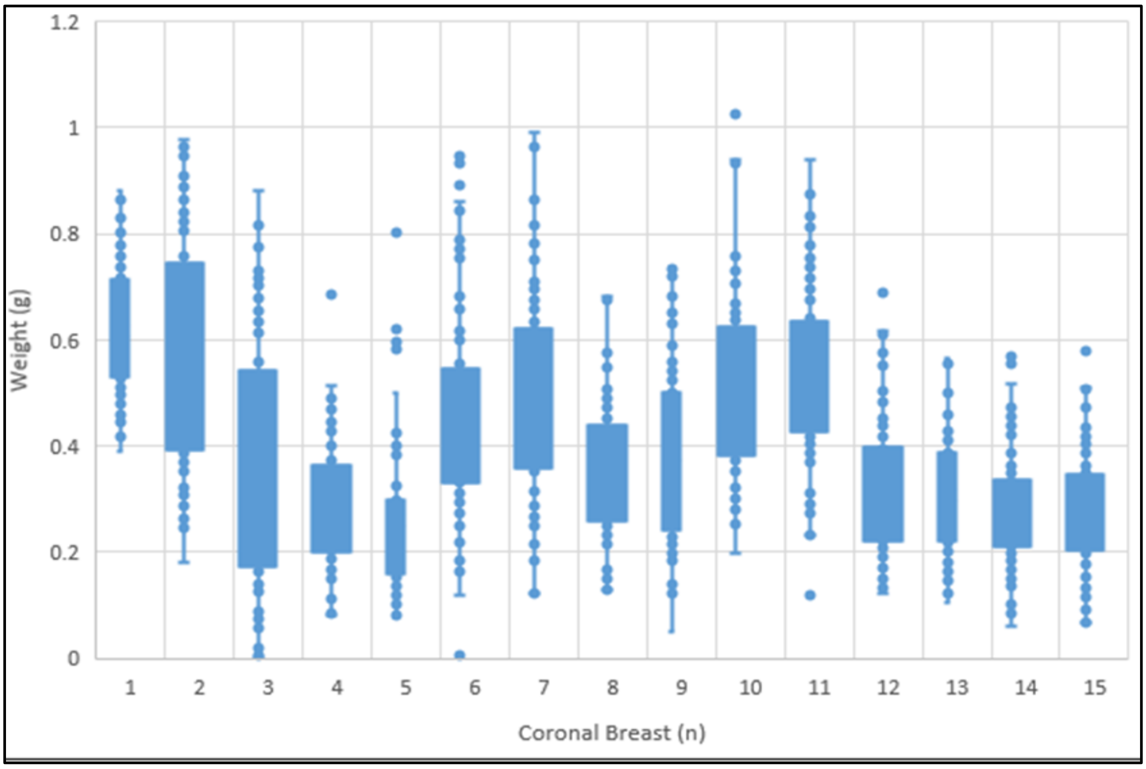

Figure 31: Adipose tissue mass of each of the 80 pockets breast dissected in the coronal plane (n=15 breasts).

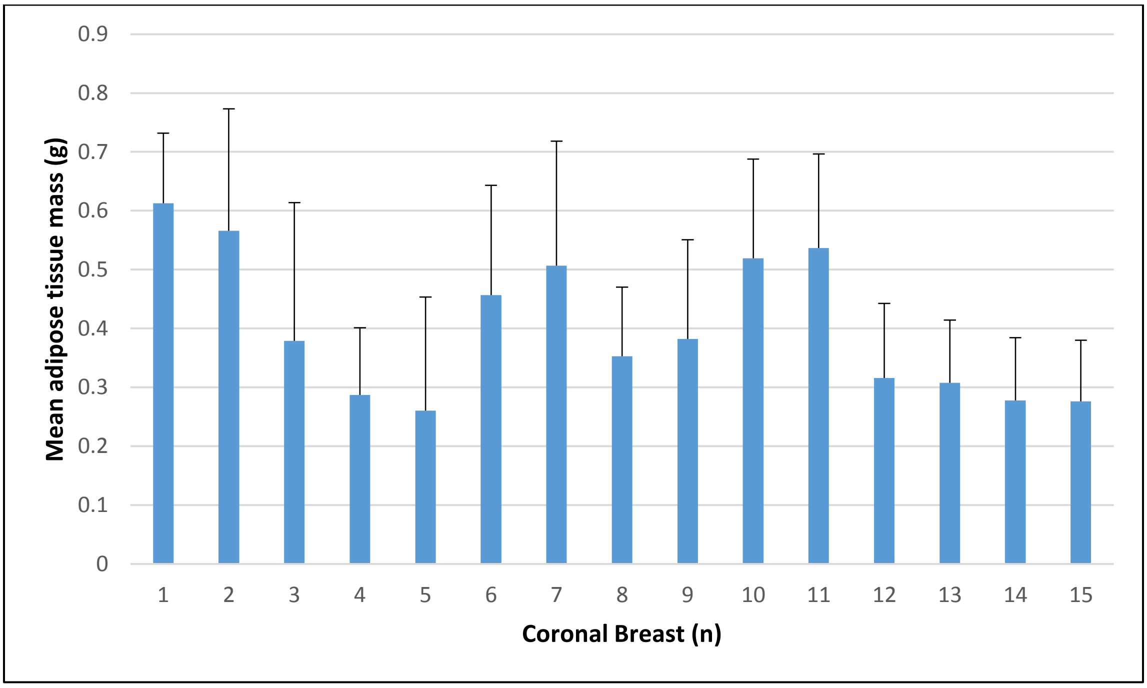

Figure 32: Mean (SD) adipose tissue mass of each pocket (n=80 pockets) per breast dissected in the coronal plane (n=15 breasts).

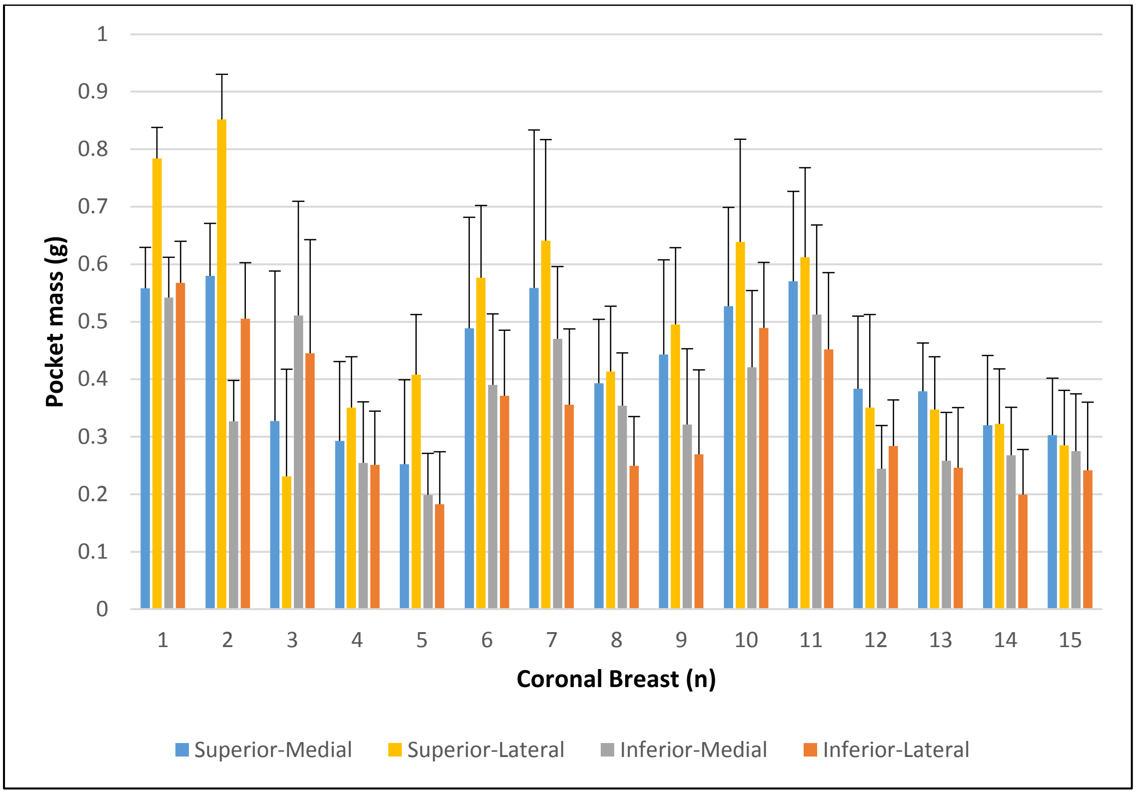

Figure 33: Mean (SD) adipose tissue mass per pocket (n=80 pockets) per quadrant of breast dissected in the coronal plane (n=15 breasts).

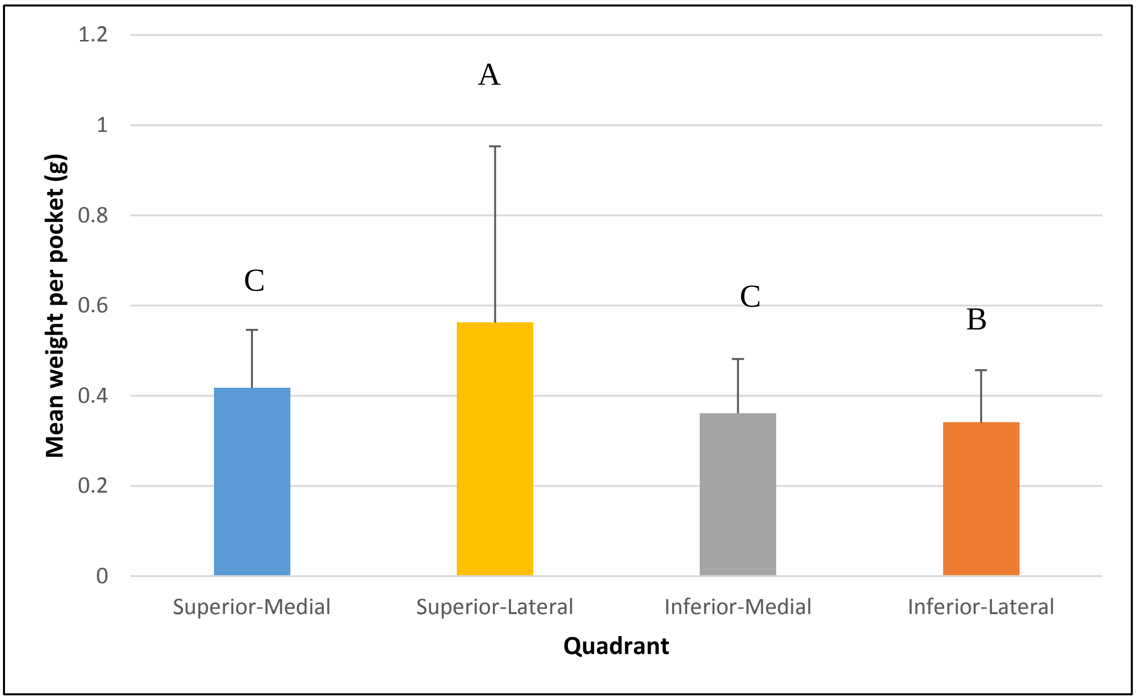

Figure 34: Mean adipose tissue mass (g) per pocket per quadrant (n=80 per breast).

[Statistical differences determined by different letters (A, B, C) whereby the quadrants with the same letter are not statistically different and the quadrants with different letters are statistically different P<0.05]

3.2.6 Surface area of adipose tissue pockets

The pocket size expressed by the surface area (length and width measurement) of each of the 80 pockets (20 pockets per quadrant per breast) measured in each of the 15 breasts dissected in the coronal plane is displayed in Figure 35. The overall mean surface area per pocket of all of the pockets measures (n=15 breasts x 80 pockets per breast) was 0.88±0.37 cm^2 (range: 0.31-1.97 cm^2) (Figure 36). Pocket size as expressed by the mean surface area of the pockets was not found to vary between the quadrants ($P>0.05$). The mean pocket surface area was the significantly greater in the superior-medial quadrant (1.05±0.42 cm^2), followed by the superior-lateral (0.9±0.37 cm^2), inferior-medial (0.79±0.35 cm^2) and inferior-lateral quadrant (0.77±0.35 cm^2; Figure 37 and Figure 38).

3.2.7 Pocket mean surface area in relation to breast surface area and volume

Consistent with pocket size expressed as pocket mass, mean pocket size expressed as pocket surface area was not found to vary with breast volume or surface area. A very low negative correlation was found between the mean adipose pocket surface area per pocket and total breast volume (r^2=- 0.1758) and total breast surface area (r^2=-0.1427; $P<0.01$; Figure 39 and Table 8). Furthermore, no significant difference found in the mean pocket surface area of the largest breasts by volume (upper tertile) compared to the smallest breasts by volume (lower tertile) $P>0.05$; (Table 8).

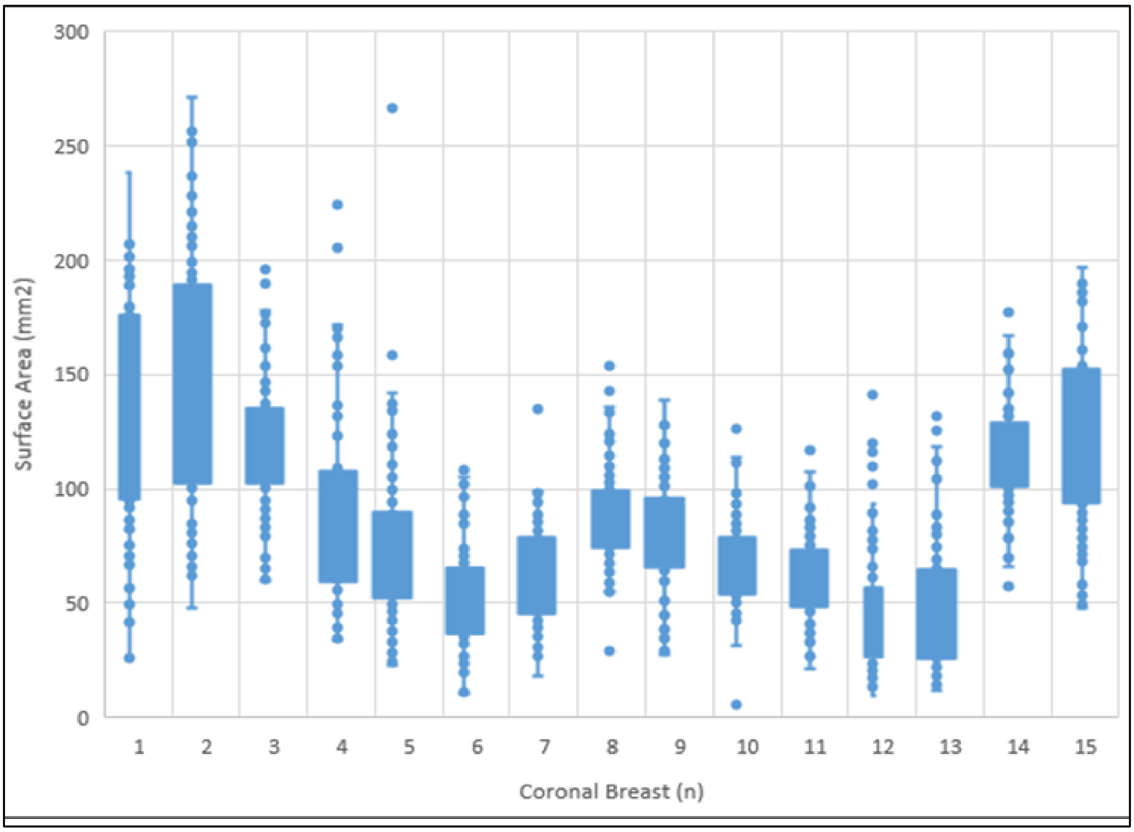

Figure 35: Surface area per pocket of the 80 pockets measured in each breast dissected in the coronal plane (n=15 breasts).

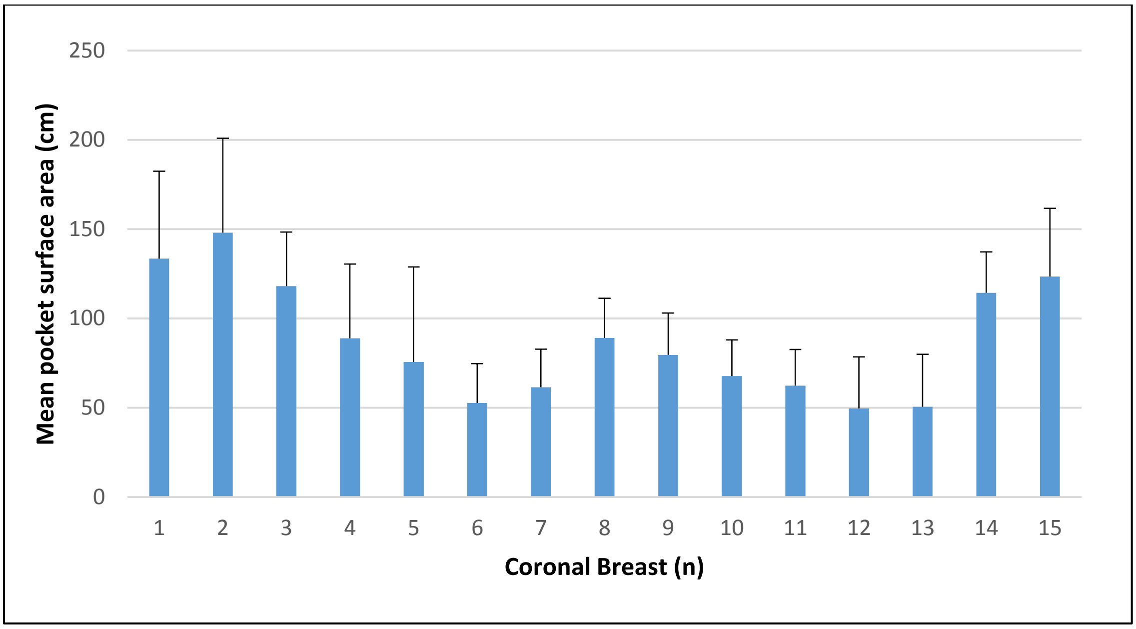

Figure 36: Mean adipose tissue pocket surface area (n=80 pockets) measured in each breast dissected in the coronal plane (n=15 breasts).

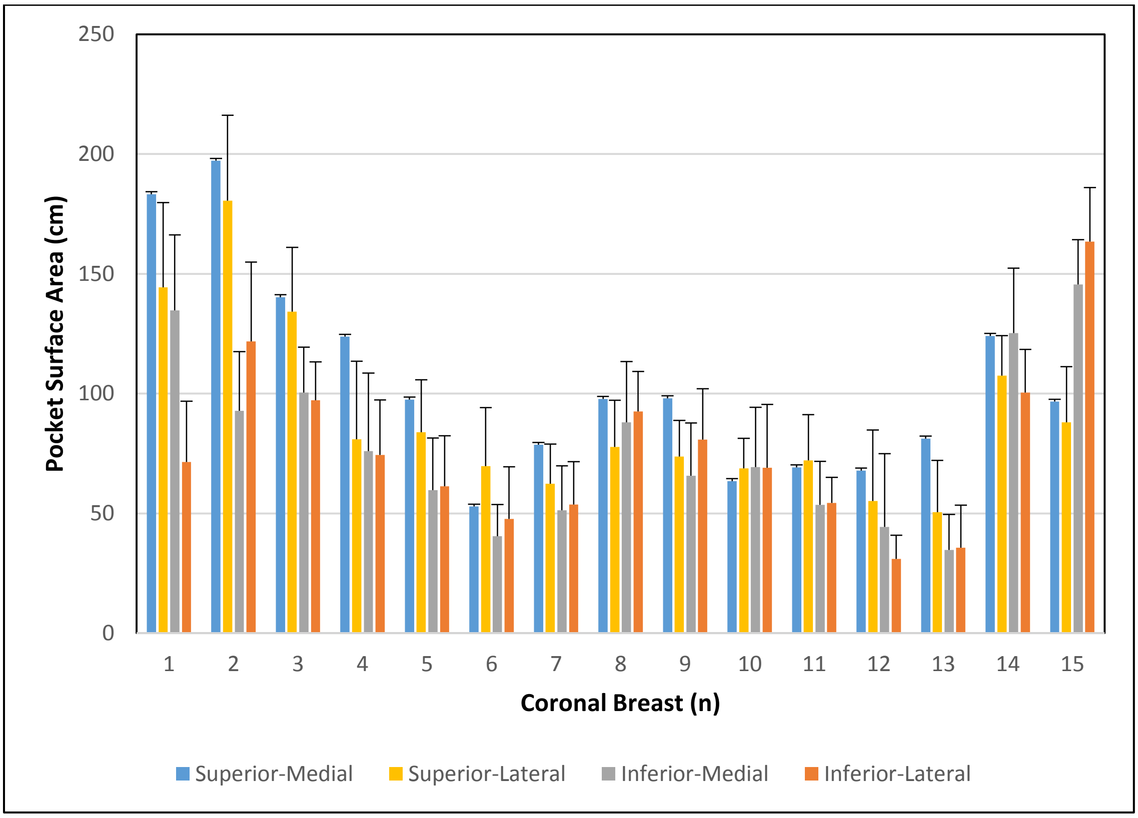

Figure 37: Mean surface area of each pocket (n=80 pockets) per quadrant measured in each breast dissected in the coronal plane (n=15 breasts).

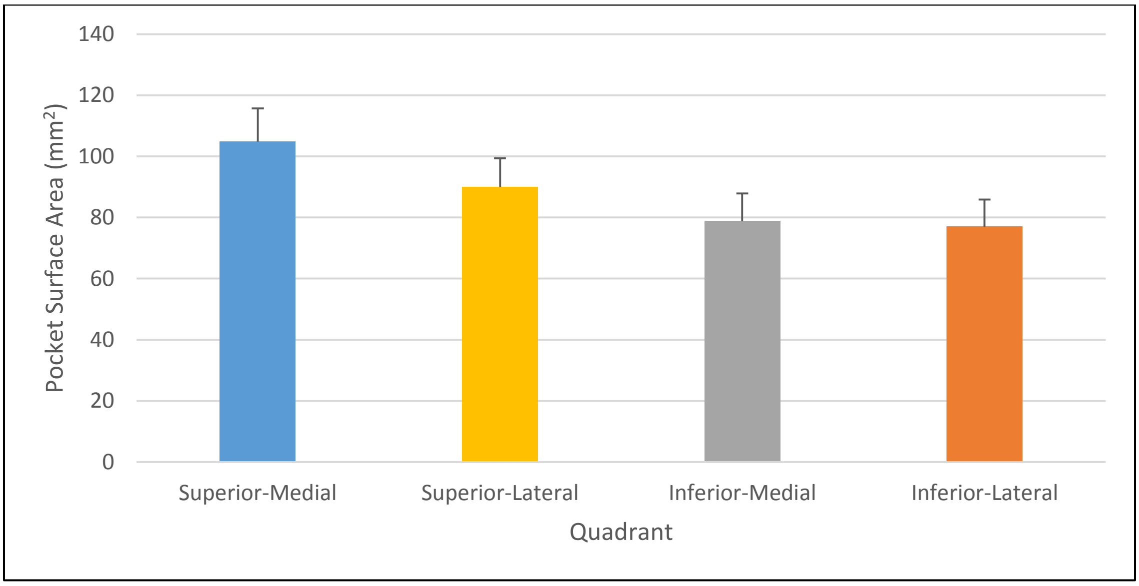

Figure 38: Mean surface area of the adipose tissue pockets per quadrant.

There is no statistical difference between the quadrants.

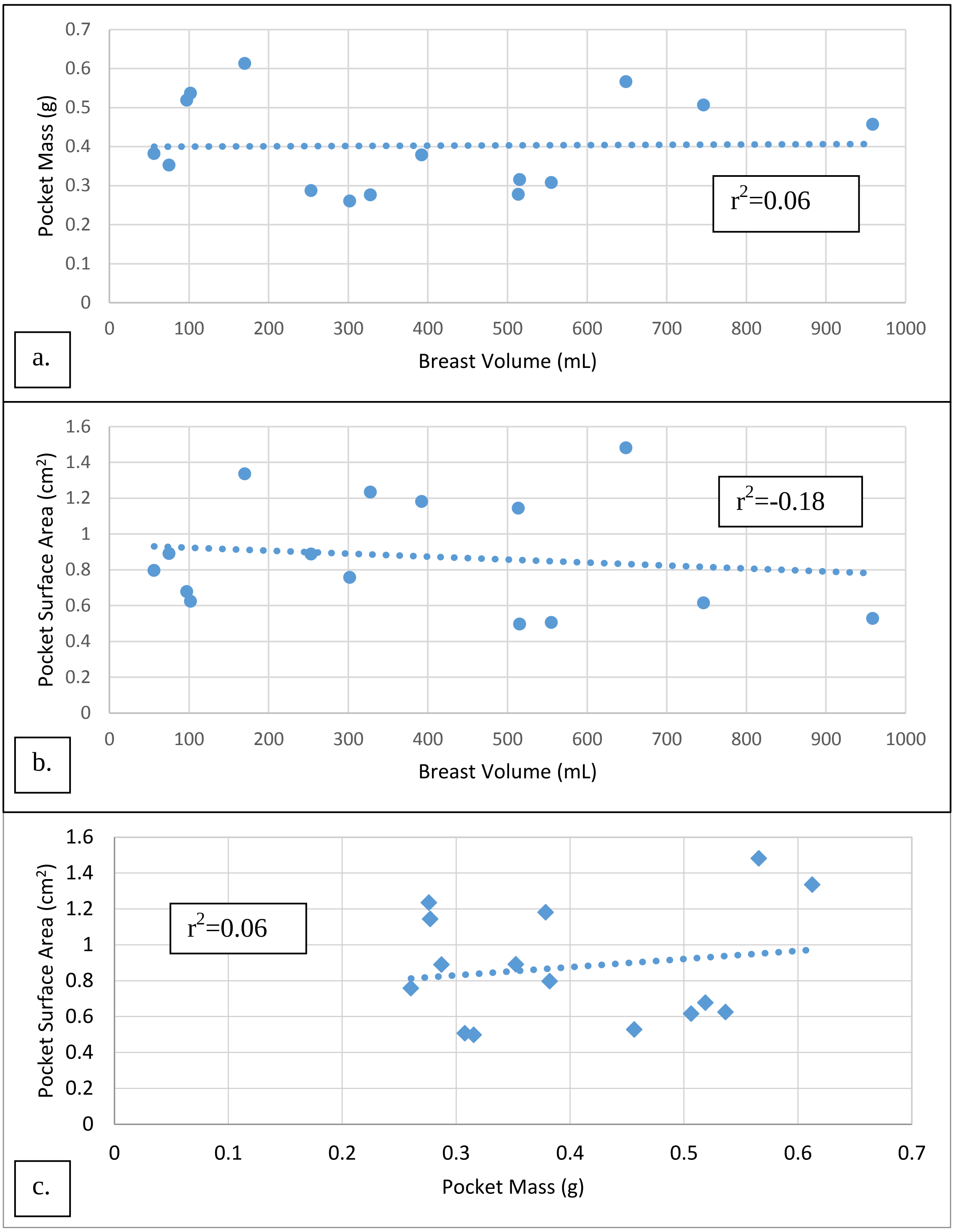

Figure 39: Correlation graphs of (a) Pocket mass to total breast volume, (b) Pocket surface area to total breast volume and (c) Pocket surface area to pocket mass per breast (n=15) (P<0.01).

3.2.8 Sagittal Qualitative Measures

Macroscopically the sagittal slices had six (6) observable tissue layers running superficial to deep; skin, superficial fibro-adipose tissue, fibro-glandular tissue, deep fibro-adipose tissue, muscle and bone. Amongst the slices, there was some variation in the thickness of each layer but consistently in all 18 slices, the superficial fibro-adipose tissue layer was observed visually to be the thickest (n=18).

The fibro-glandular layer was consistently found in all six slices found to be thicker (>1 cm thick) in the midline slice compared to the more medial and lateral para-sagittal slices. No glandular tissue was seen in the most medial slices (n=6), which consisted of very small fibro-adipose pockets bound within tight fibrous strands. Some glandular tissue was seen in the most lateral slices (<1cm thick), which were embedded in a large cluster of fibro-adipose tissue pockets.

In contrast to the coronal dissection a clear breast capsule was not evident macroscopically in any of the sagittal slices. A thin layer of fascia was observed however running parallel to the skin through the superficial fibro-adipose tissue, which had multiple fibrous tissue bands connecting this layer of fascial tissue to the dermis. This was consistent with Cooper’s Anterior Extensions of the Anterior Lamellae. A thin layer of fascia was not observed however running parallel to the skin posterior to the gland. Multiple fibrous tissue bands connecting posterior surface of the gland to the pectoralis major muscle fascia were observed. This was consistent with Cooper’s Posterior Extensions of the Posterior Lamellae. The Anterior Extensions of the Anterior Lamellae were consistently thicker than the Posterior Extensions of the Posterior Lamellae. The Posterior Extensions of the Posterior Lamellae were very numerous but were easy to separate using blunt dissection.

The three-dimensional fibro-adipose structure when viewed from the sagittal aspect had two distinct sections, one anterior to the gland and one posterior to the gland (Figure 40). Fibro-adipose pockets were evident anterior and posterior to the gland on all of the surfaces of the sagittal slices (Figure 40). The pockets anterior to the gland were larger and less numerous compared to the pockets posterior to the gland. The collagen of the fibro-adipose tissue anterior to the gland could be traced superficially to the dermis (Anterior Extensions of the Anterior Lamellae). The collagen of the fibro-adipose tissue posterior to the gland could be traced posteriorly to the pectoral fascia (posterior extensions of the posterior lamellae) in an organised pattern. Posterior to the gland, the adipose tissue pockets appeared to be smaller but more numerous compared to the fibro-adipose pockets anterior to the gland. The posterior layer of fibro-adipose tissue varied in depth across the different slices, however in all of the sagittal breast slice surfaces, the posterior layer was thicker in the mid-nipple slices compared to the medial and lateral slices. The posterior layer in the mid-nipple slices were consistently >1 cm, whereas the medial and lateral slices consistently <1 cm thick.

Evidence of perimeter attachments of the breast to the chest wall were evident on the sagittal breast slices. The superior perimeter attachment of the breast was observed in four of the 18 slice surfaces, connecting the superior perimeter of the breast to the inferior portion of the clavicle. An inferior perimeter of the breast connecting to either the 5^{th} or 6^{th} rib was observed in six of the 18 sagittal slices. No lateral or medial perimeter attachments were observed in the sagittal breast slices. The lateral confluence was only observed on one slice and it was not connected to bone.

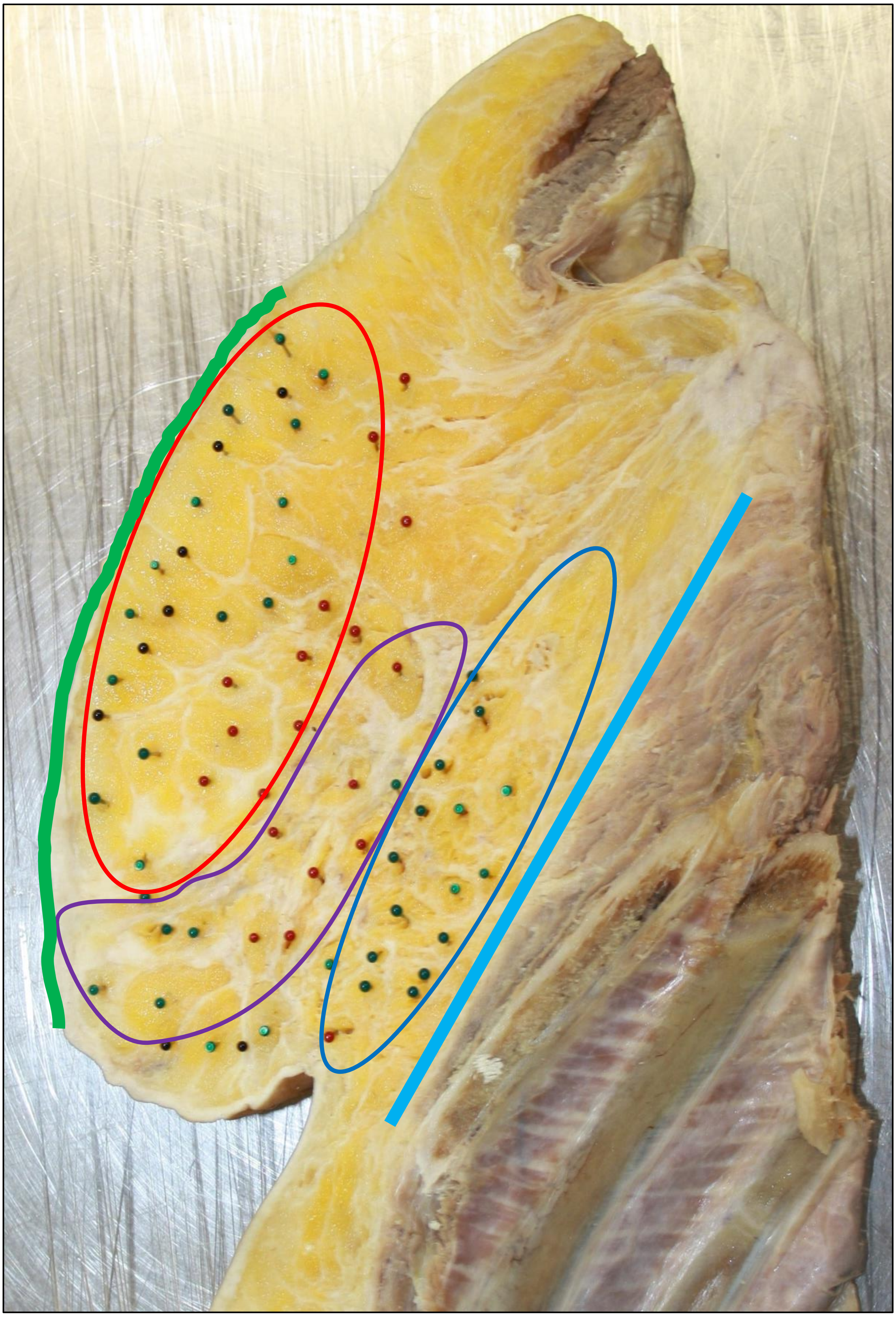

Figure 40: Mid-sagittal slice of the breast through the nipple showing the dermis (green) and the Superficial Pectoral fascia (light blue). The regional depths are circled anterior to the gland (red), within the gland (purple) and posterior to the gland (dark blue).

3.2.9 Number (n) of pockets anterior and posterior to the gland

The mean number of fibro-adipose pockets located anterior and posterior to the gland of 18 sagittal breast slice surfaces (six breast slice surfaces in each of the three breasts; Figure 41 and Figure 42) is displayed in Table 9. In all 18 breast slices, the number of fibro-adipose pockets found anterior to the gland was less in number (35±16, range: 20-49) compared to the number found posterior to the gland (53±31, range: 36-78).

3.2.10 Length of the anterior extensions of the anterior lamellae

The mean length of the 180 number collagen fibres measured that connected the superficial fibro-adipose cobweb to the dermis (Anterior Extensions of the Anterior Lamellae) for the 18 breast slice surfaces (six breast slice surfaces in each of the three breasts) examined in the sagittal plane is shown in Table 9 and Figure 43. The mean length across the 18 breast slice surfaces was 19±5 mm (range: 9-34 mm). The Anterior Extensions were longer at the mid-nipple sagittal breast slices (23.5 mm) compared to the Anterior Extensions measured at the medial and lateral sagittal breast slices (13.5 mm and 19 mm respectively; Figure 44**).**

3.2.11 Pocket surface area (mm^2)

The surface area of the fibro-adipose pockets (minimum 20 pockets measured per sagittal breast slice surface both anterior and posterior to the gland) measured anterior and posterior to the gland microscopically in the sagittal plane is displayed in Table 9. The surface area of the fibro-adipose pockets located anterior to the gland in all of the 18 breast slices were consistently larger (mean: 0.89±0.32 cm^2, range: 0.34-1.20 cm^2) than those located posterior to the gland (mean: 0.33±0.22 cm^2, range: 0.22-0.87 cm^2).

Table 9: Mean surface measurements in the sagittal plane breast slices (n=18 slices, n=3 breasts).

	Mean length of anterior extension (mm) (SD)	Mean no pockets (n) (SD)		Mean pocket surface area (mm^2) (SD)	
		Anterior to gland	Posterior to gland	Anterior to gland	Posterior to gland
Medial 1	13 (1.5)	20 (1.5)	32 (9)	0.6 (0.24)	0.19 (0.05)
Medial 2	14 (1.7)	24 (3.2)	29 (7.6)	0.62 (0.24)	0.17 (0.05)
Mid-Nipple 1	25 (2.3)	55 (1.3)	93 (12.4)	1.2 (0.23)	0.57 (0.26)
Mid-Nipple 2	22 (2.1)	53 (14)	94 (21)	1.27 (0.25)	0.55 (0.28)
Lateral 1	20 (1)	30 (7.8)	35 (9.6)	0.87 (0.18)	0.24 (0.11)
Lateral 2	19 (5)	31 (9)	37 (9)	0.8 (0.17)	0.27 (0.11)
Mean	19 (5)	35 (16)	53 (31)	0.89 (0.32)	0.33 (0.22)

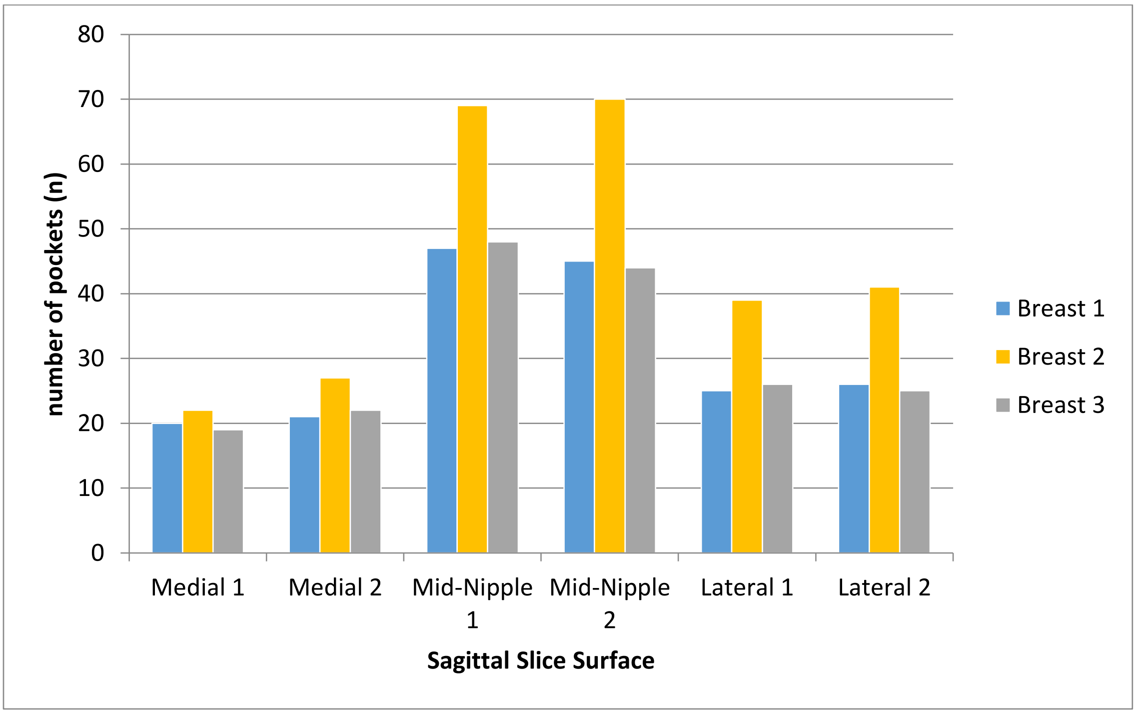

Figure 41: Number of fibro-adipose tissue pockets anterior to the gland per breast measured in the sagittal breast slice (n=18 slices, n=3 breasts).

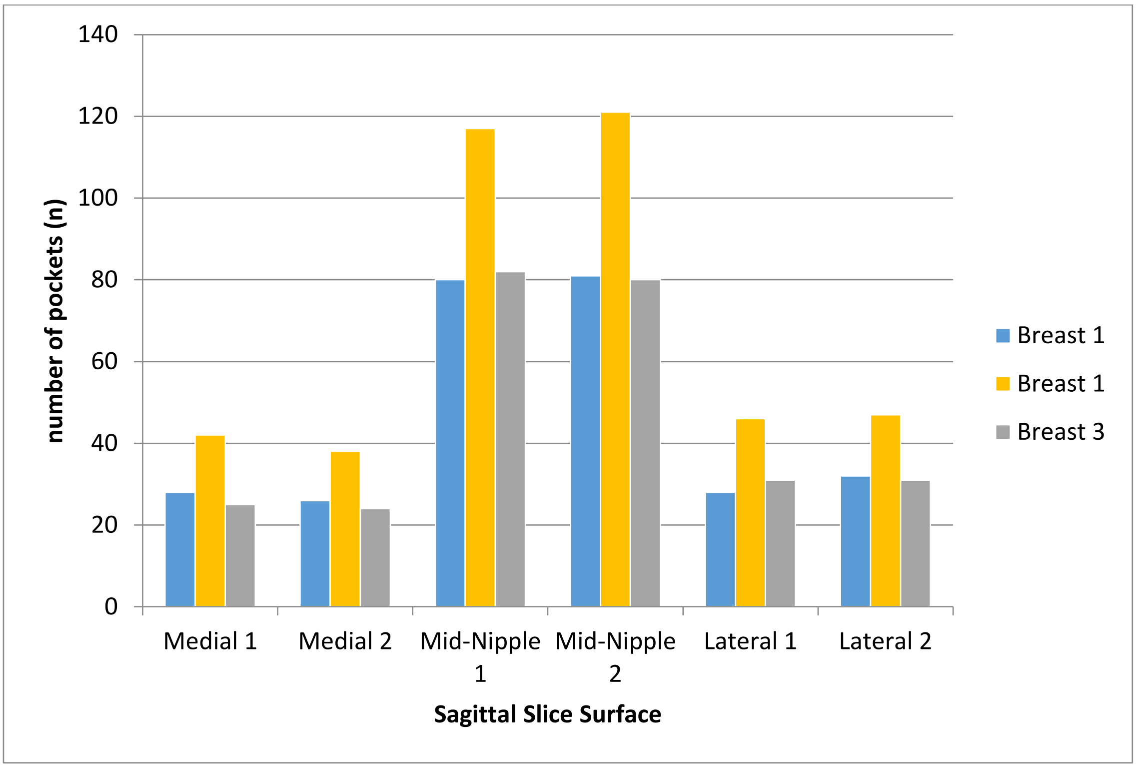

Figure 42: Number of fibro-adipose tissue pockets posterior to the gland per breast measured in the sagittal breast slice (n=18 slices, n=3 breasts).

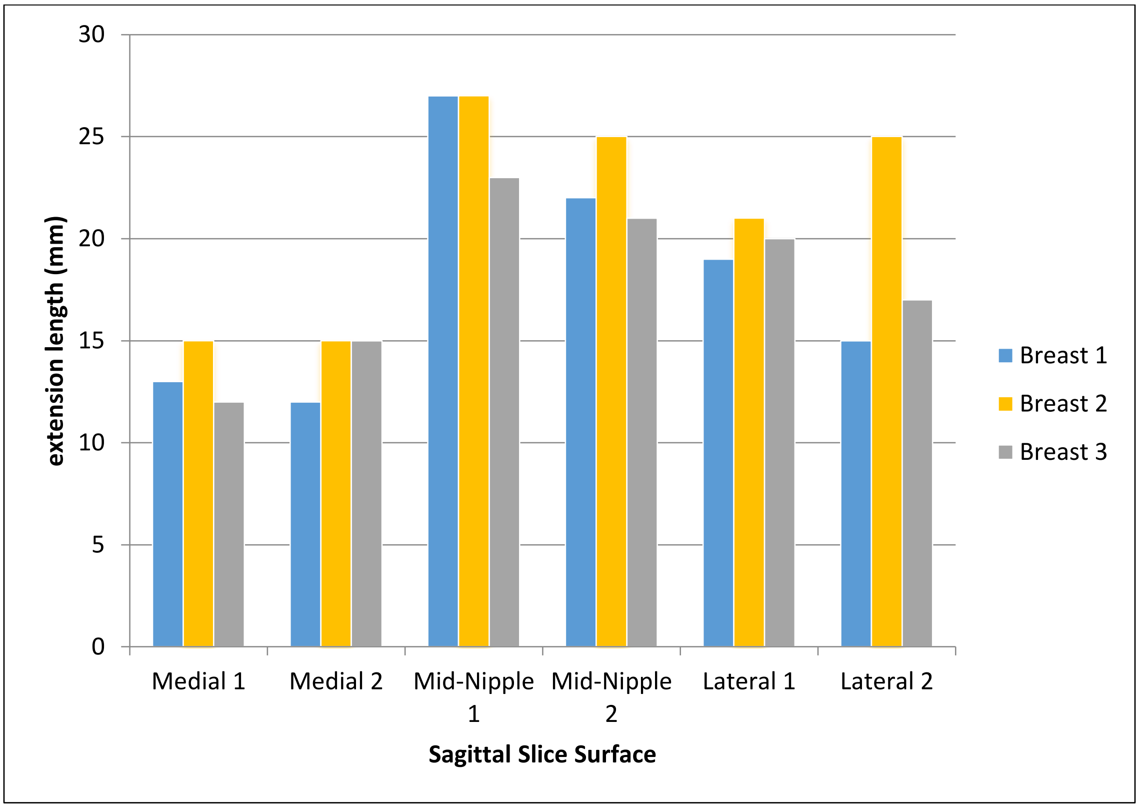

Figure 43: Anterior Extension of the Anterior Lamellae length per breast measured in the sagittal breast slice (n=18 slices, n=3 breasts).

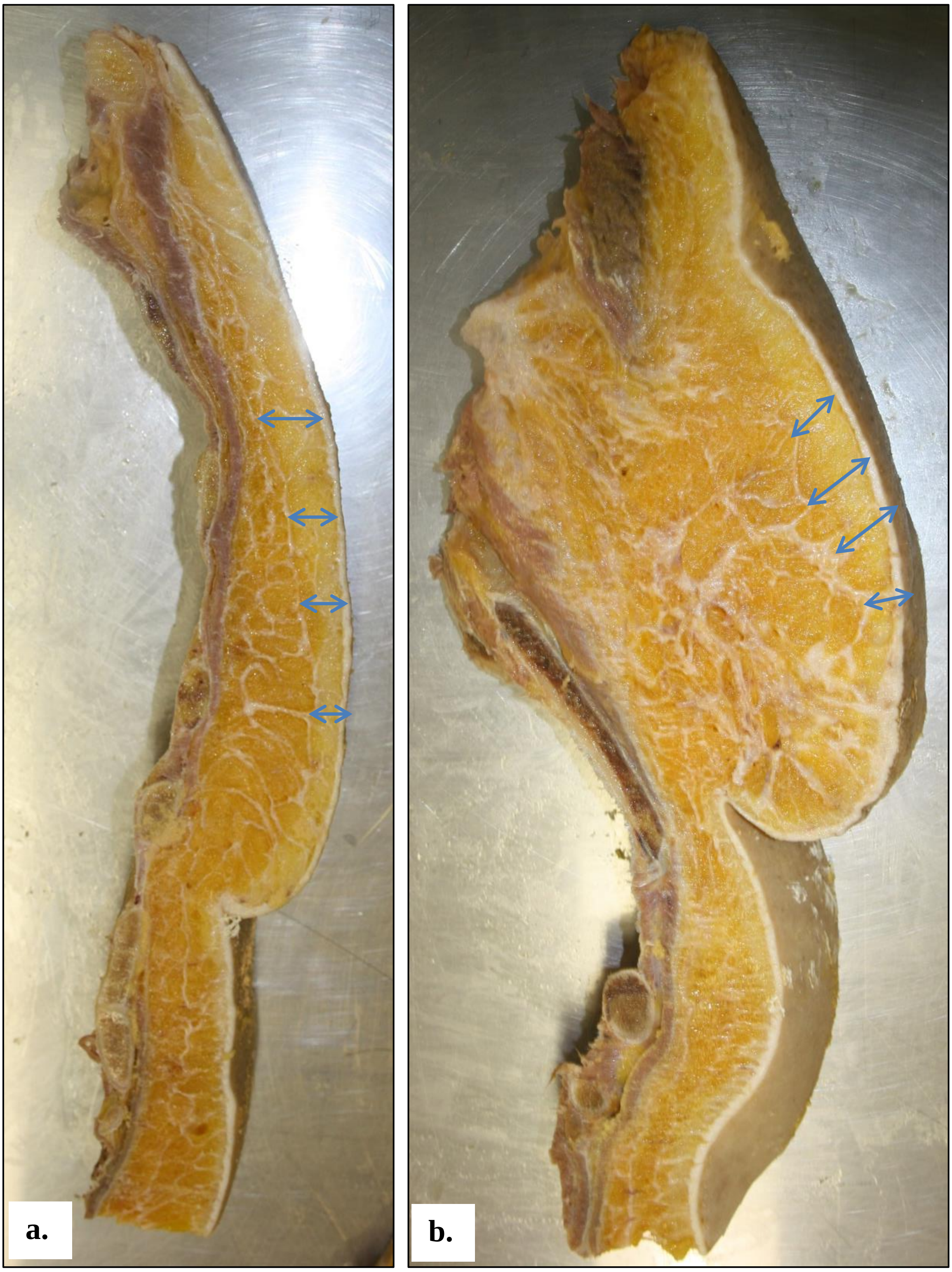

Figure 44: Anterior Extension of the Anterior Lamellae length on the a) Medial and b) Lateral para-sagittal breast slices.

3.3 Attachment of the breast to the chest wall

Posterior Attachment

The posterior surface of the breast was found to attach to the superficial pectoral fascia and to a variable degree the superficial fascia of serratus anterior, external oblique and rectus abdominus muscles by many small fibrous bands that house small adipose pockets in a similar fashion to the superficial fibro-adipose structure Cooper referred to as the Posterior Extensions of the Posterior Lamellae (Figure 44 and Figure 45). These attachments were found to be numerous and very weak (i.e. were easily dissected with blunt dissection) compared to the perimeter attachments (which required sharp dissection). They also extended beyond the surface area of the gland and therefore beyond what Cooper referred to as the “Posterior Lamellae”. A posterior attachment of the breast was consistently found in all 12 breasts to span from the nipple to the superficial pectoralis major muscle fascia forming a horizontal septum which divided the breasts in to a superior 2/3 and inferior 1/3. The horizontal septum required only blunt dissection in all 12 breasts and had no observable periosteal attachment. The same horizontal septum was also identified in the sagittal plane dissections where it was also observed to span from the nipple to the anterior aspect of the chest wall to attach onto the superficial pectoralis major muscle fascia (Figure 45).

Perimeter Attachments

The perimeter attachment (Figure 47) of the breasts was consistently found (in all ten breasts dissected in the coronal plane to this depth) to be stronger than the posterior wall attachments, which only required blunt dissection.

In all 12 coronal dissections, the **superior arc attachment** of the breast was consistently found to attach to the anterior aspect of the chest wall onto both the superficial pectoralis muscle fascia and the deep pectoralis muscle fascia. The medial and lateral third of the superior arc perimeter attachment were consistently found to attach to the superficial pectoralis muscle fascia. The most lateral third of the superior arc attachment consistently curved in an inferior-lateral direction across the superior-lateral aspect of the pectoralis major muscle, along the superficial pectoralis muscle fascia where it merged with the lateral arc of the perimeter attachment. In a similar fashion, the most medial third curved across the medial aspect of the superficial pectoralis muscle fascia in an inferior-medial direction, where it merged with the fibres of the medial arc of the perimeter attachment. The middle third of the superior arc attachment (the apex of the curve) was consistently found to dive between the heads of the pectoralis major muscle (sternal and clavicular) and intersect with the deep pectoralis muscle fascia. This facial connection was consistently found to be deformable but strong as it required sharp dissection. Although no direct periosteal attachment of the middle third of the superior arc attachment was found, an indirect periosteal attachment to the clavicle was found via the superficial and deep pectoral fascia which were observed to traverse superiorly and attach to the clavicle.

The **medial arc attachment** of the breast was found to tightly adhere the breast to the chest wall along the lateral edge of the sternum with short fibres running from the dermis directly to the periosteum of the sternum. Little to no adipose tissue was found in this region of the perimeter, only dense, short fibres of connective tissue. The medial attachments were considered to be the strongest of the perimeter attachments due to the time required to sharply dissect this tissue in comparison to the other perimeter attachments.

The **lateral arc attachment** consisted of a sling of fascia formed by multiple irregular shaped bands of fascia from the superficial fascia of three muscles (pectoralis major, serratus anterior and external oblique muscles). Fascial bands from the superior edge of external oblique muscle formed the most inferior aspect of the lateral arc, with fibres running in a transverse direction. These fibres merged with the fascia of the anterior aspect of serratus anterior, with fibres running in a superior-lateral oblique direction. The two bands of fibres then traversed the surface of the serratus anterior muscle as two parallel fascial bands in a superior-medial direction where they intersected with the fascial from the lateral edge of the pectoralis major muscle (Figure 47). The fascial contribution from each muscle originated separately at the inferior-lateral border of the breast but had merged to form one sling, at the level of the nipple (horizontally). Superior to the nipple, the lateral arc perimeter attachment was observed to be a single sling (Lateral Fascia Confluence[36]) which traversed the lateral boarder of the pectoralis major muscle and merged with the most lateral aspect of the superior arc perimeter attachment. There was no evidence of a “Tail of Spence” as described by Cooper [1]. In contrast to the medial attachment, the lateral attachment was found to consist of longer fibrous strands, which were interspersed with larger lobules of adipose tissue within the axilla.

The **inferior arc attachment** of the breast, also known as the Inframammary Fold (IMF) was found to be a tightly adhered band of tissue anchoring the inferior portion of the breast strongly to the superficial fascia of the anterior chest wall (superficial fascial pectoralis major, external oblique and rectus abdominus muscles). From the sagittal breast slices there appears to be some space in which direct connections may exist between the fibro-adipose tissue of the breast (posterior extensions of the posterior lamellae) and the 5th rib in the most breast slices. This occurs in the space between the inferior border of the pectoralis major muscle and the superior border of the rectus abdominus muscle and external oblique muscle fibres (Figure 45i-iii). The medial third of the IMF was found to arc in a superior-medial oblique direction to the sternum where it merged with the fibres of the medial arc attachment. The lateral third of the IMF was seen to merge with the fibres of the lateral perimeter (what Matousek et al, 2014 referred to as the Lateral Fascial Confluence [36]).

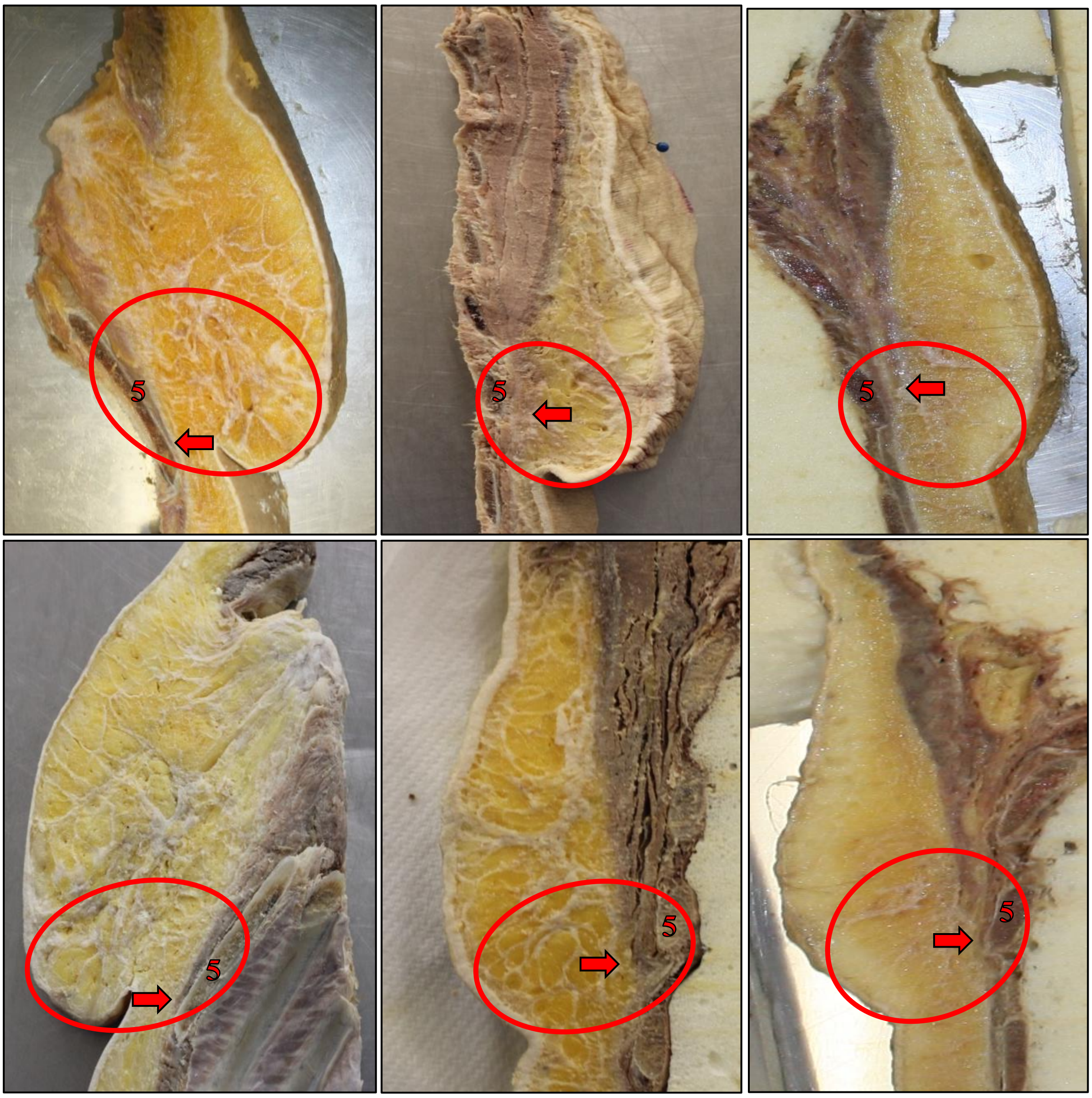

Figure 45i Mid-sagittal slice of the female breast through the nipple with the 5th rib marked (5), the inferior border of pectorals major muscle marked (arrow) and the region of the Triangle Fascial Condensation [41] circled.

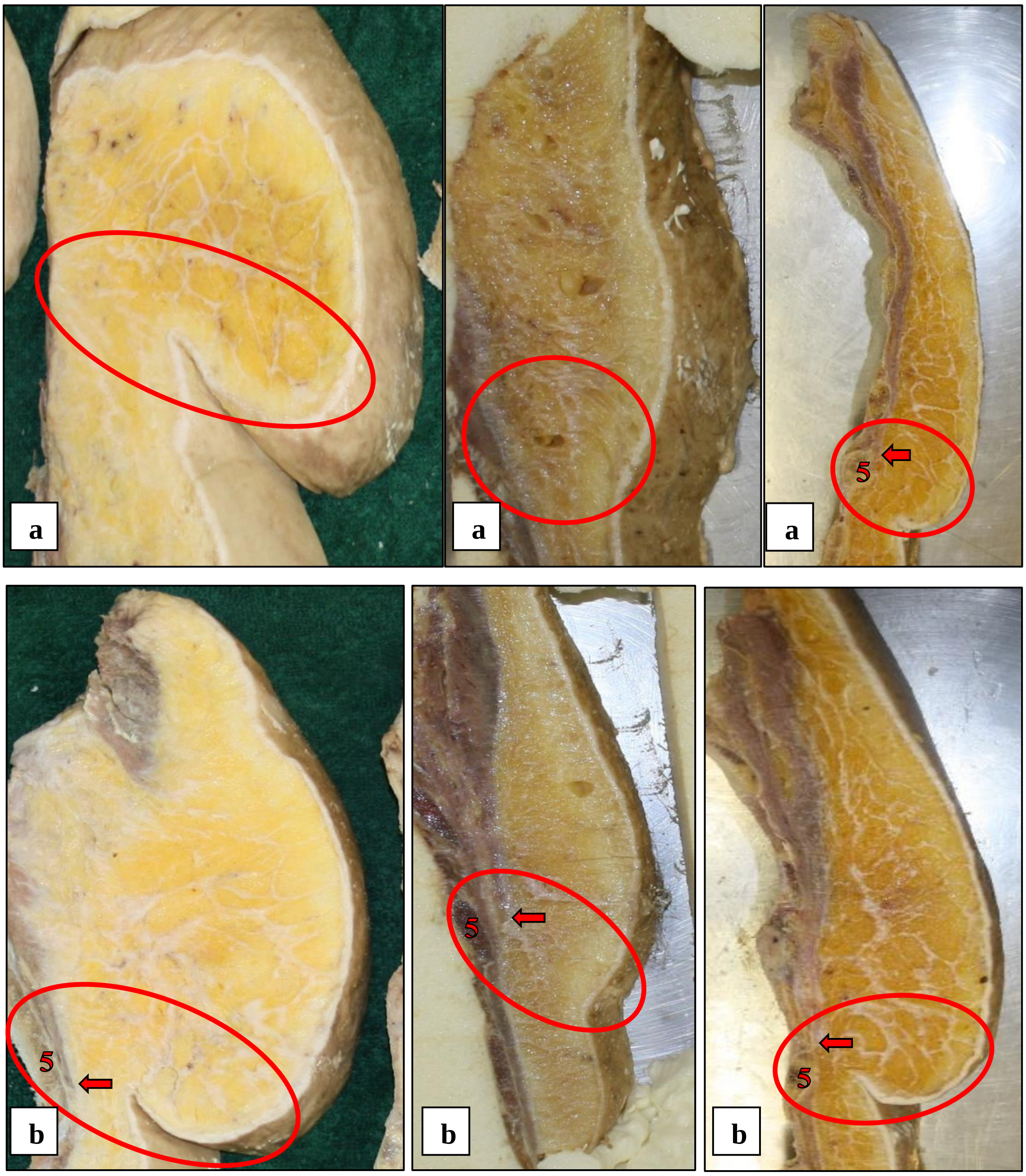

Figure 45ii Sagittal slice of the female breast through the lateral edge of the areola (a) and lateral breast boarder (b) with the 5th rib marked (5 – where visible), the inferior border of pectorals major muscle marked (arrow- where visible) and the region of the Triangle Fascial Condensation [41] circled.

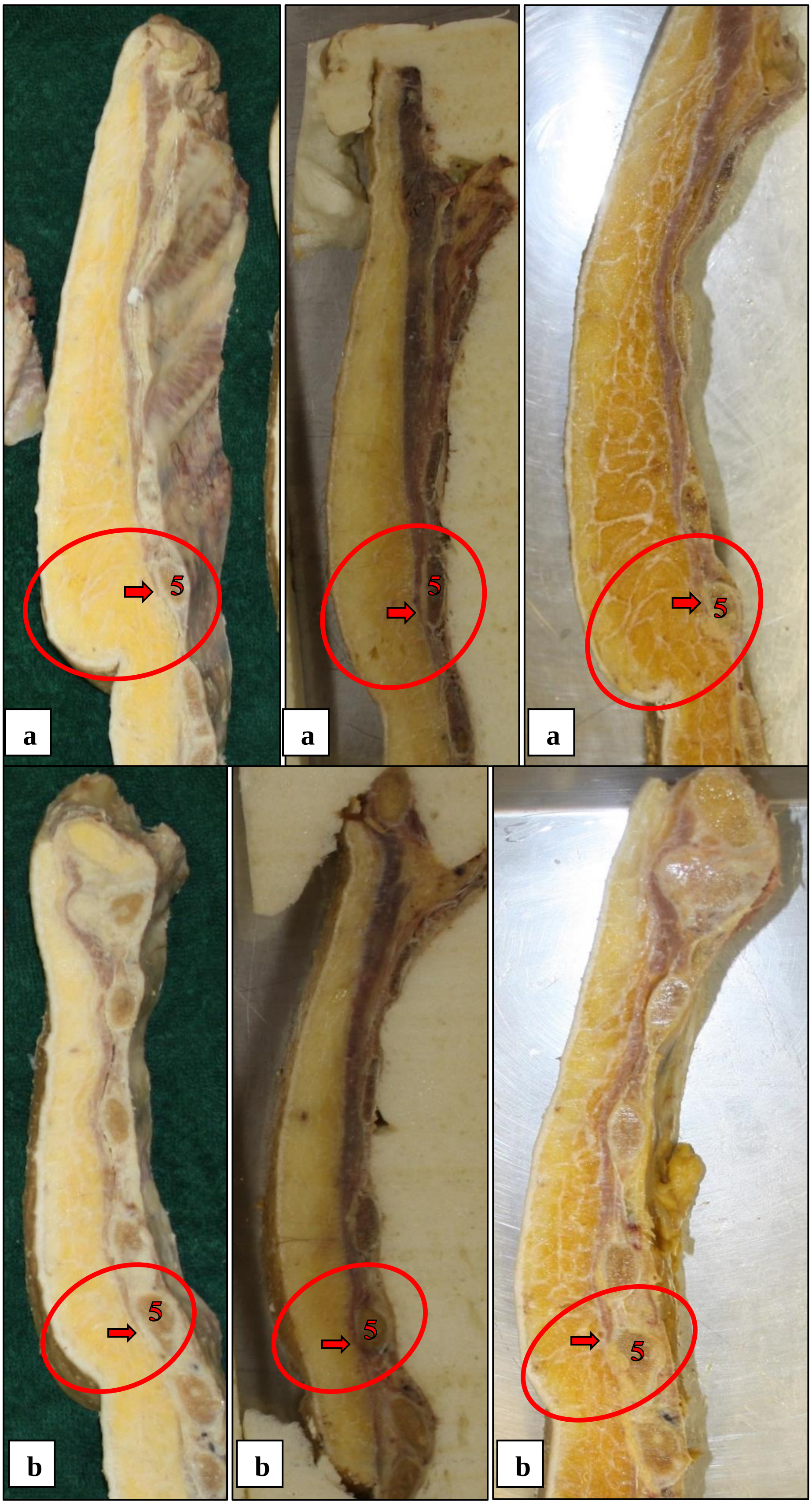

Figure 45iii Sagittal slice of the female breast through the medial edge of the areola (a) and medial breast boarder (b) with the 5th rib marked (5), the inferior border of pectorals major muscle marked (arrow) and the region of the Triangle Fascial Condensation[41] circled.

3.3.1 Perimeter: Regional Anatomy

From the coronal plane dissection of the perimeter (at the depth where the fibro-adipose and fibro-glandular tissues had been removed), the perimeter of the breasts were consistently observed in the 12 breasts dissected to this depth to have an irregular and uneven circular shape (Figure 47 and Figure 49). In 10 of the 12 breasts dissected to this level, the apex of the superior perimeter (middle one third) was located between the sternal and clavicular heads of the pectoralis major muscle, with the rest of the superior perimeter attaching to the superficial fascia of pectoralis major muscle. In the remaining two of the 12 breasts, the superior perimeter was located 2-3cm inferior to the division of the sternal and clavicular heads of the pectoralis major muscle.

Once the superficial chest wall muscles were removed, the location of the perimeter relative to the ribs revealed that the most superior aspect of the “circle” shaped perimeter was located within the 3rd intercostal space, in vertical alignment with the mid clavicular line. The most inferior aspect of the “circle” of the perimeter was also consistently in vertical alignment with the mid-clavicular line in all of the twelve breasts, however the location of the inferior aspect of the “circle” of the perimeter relative to the ribs was found to vary. In eight of the 12 breasts, it was level with the sixth rib, in three it was level with the fifth rib and in one of the breasts there was no visible bony attachment of the inferior perimeter of the breasts.

The medial aspect of the curvature of the perimeter was consistently found to be in contact with the lateral boarder of the sternum at the level of the 4th intercostal space. The lateral curvature of the perimeter of the breasts were found to be have two distinct parts, one slightly more lateral and superior (lateral/superior sling) than the other (medial/inferior sling). The lateral/superior slings were consistently found to follow an arc along the mid-axillary line and the medial/inferior slings were consistently found to follow the inferior boarder of the pectoralis major and the serratus anterior muscle boarders, to meet the inferior aspect of the perimeter of the breast.

3.3.2 Perimeter: Quantitative Data

The perimeter of the 10 breasts measured with the surgical sill in the coronal plane is displayed in Figure 46, Figure 47 and Table 10. The mean perimeter of the 10 breasts was 46.01±12.2 cm (range: 32-60 cm).

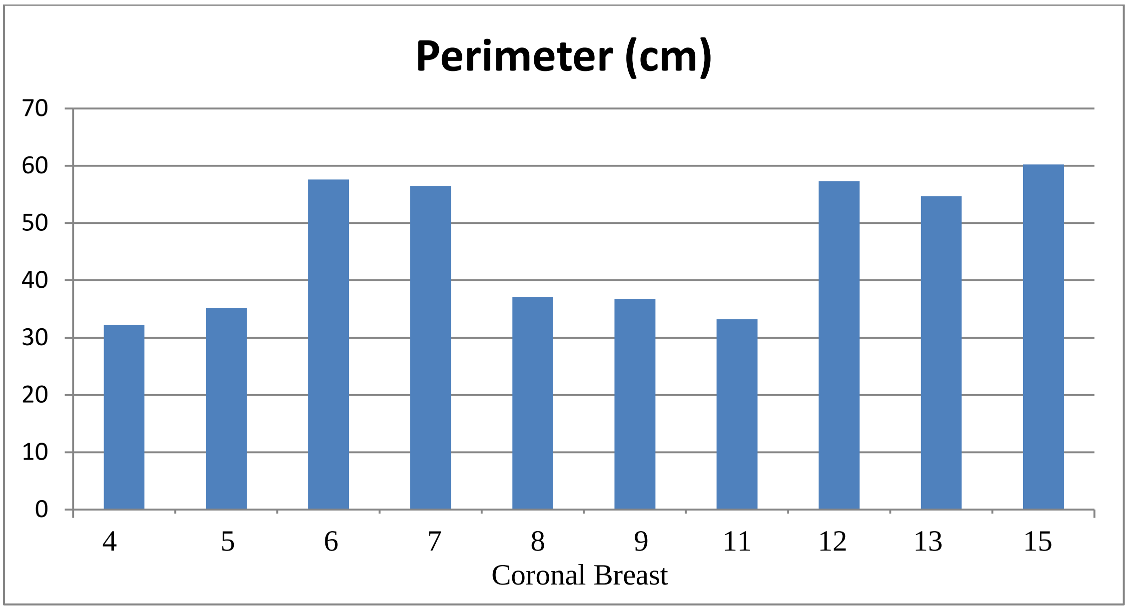

Figure 46: Breast perimeter per breast.
[Coronal Breast 1, 2, 3, 10 and 14 were not examined]

Table 10: Breast perimeter and the surface area contribution of each muscle within the perimeter.

Coronal Breast	Volume (mL)	Perimeter (cm)	Pectoralis Major (%)	Serratus Anterior (%)	Rectus Abdominus (%)	External Oblique (%)
1	169.98	-	100	0	0	0
2	649.03	-	95	0	5	0
4	253.32	32.2	56	16	13	15
5	301.76	35.2	72	9	6	13
6	958.70	57.6	89	11	0	0
7	746.31	56.5	86	14	0	0
8	74.95	37.1	85	0	0	15
9	56.071	36.7	80	7	0	13
11	101.84	33.2	63	24	9	3
12	515.43	57.3	77	14	9	0
13	555.22	54.7	77	14	9	0
15	327.84	60.2	91	9	0	0
Mean	393	46	81	10	4	5

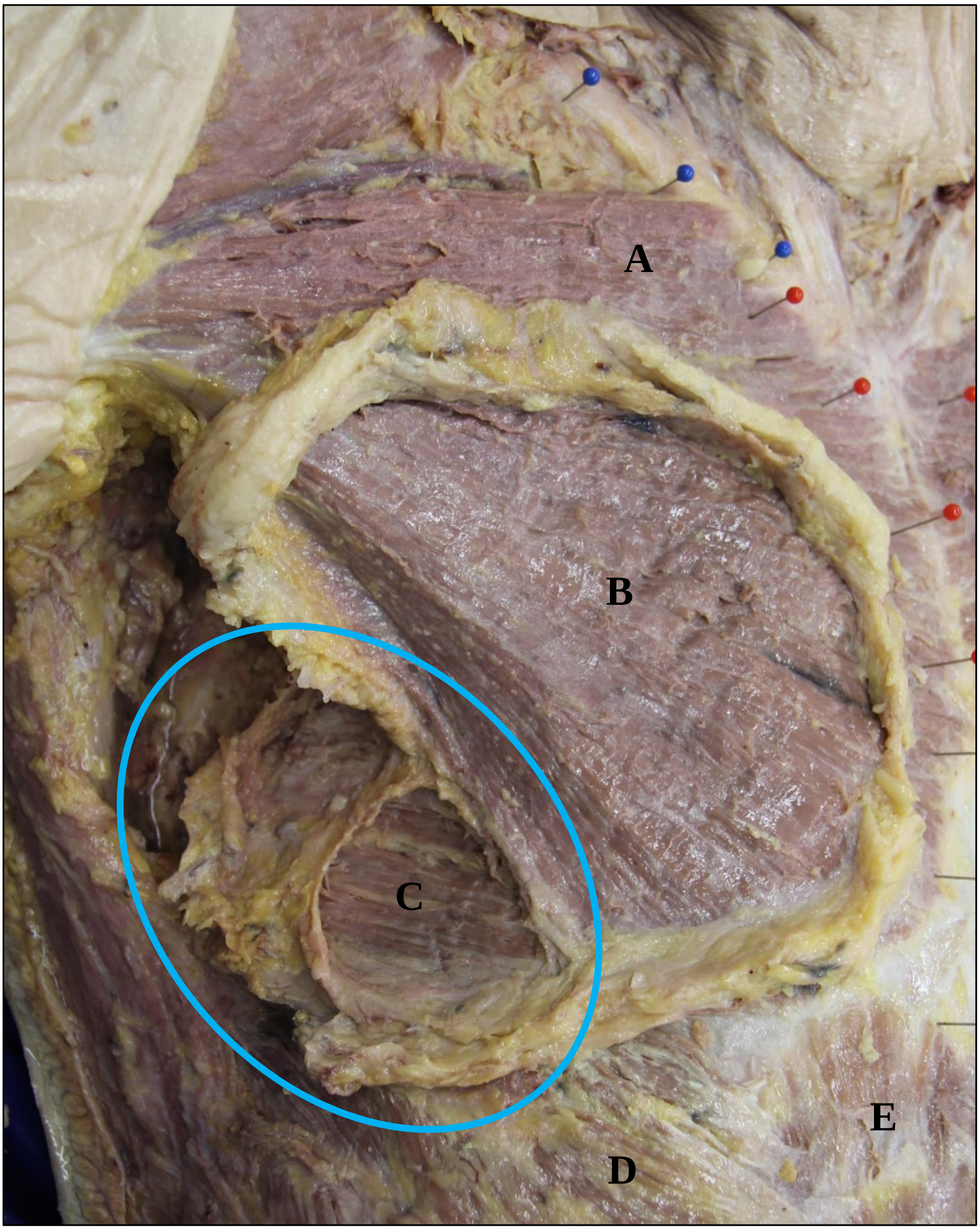

Figure 47: Perimeter of the breast, with the lateral fascia confluence circled. A) Pectoralis Major muscle (clavicular head), B) Pectoralis Major muscle (sternal head), C) Serratus Anterior mucles, D) External Oblique muscle, E) Rectus Abdominus muscle.

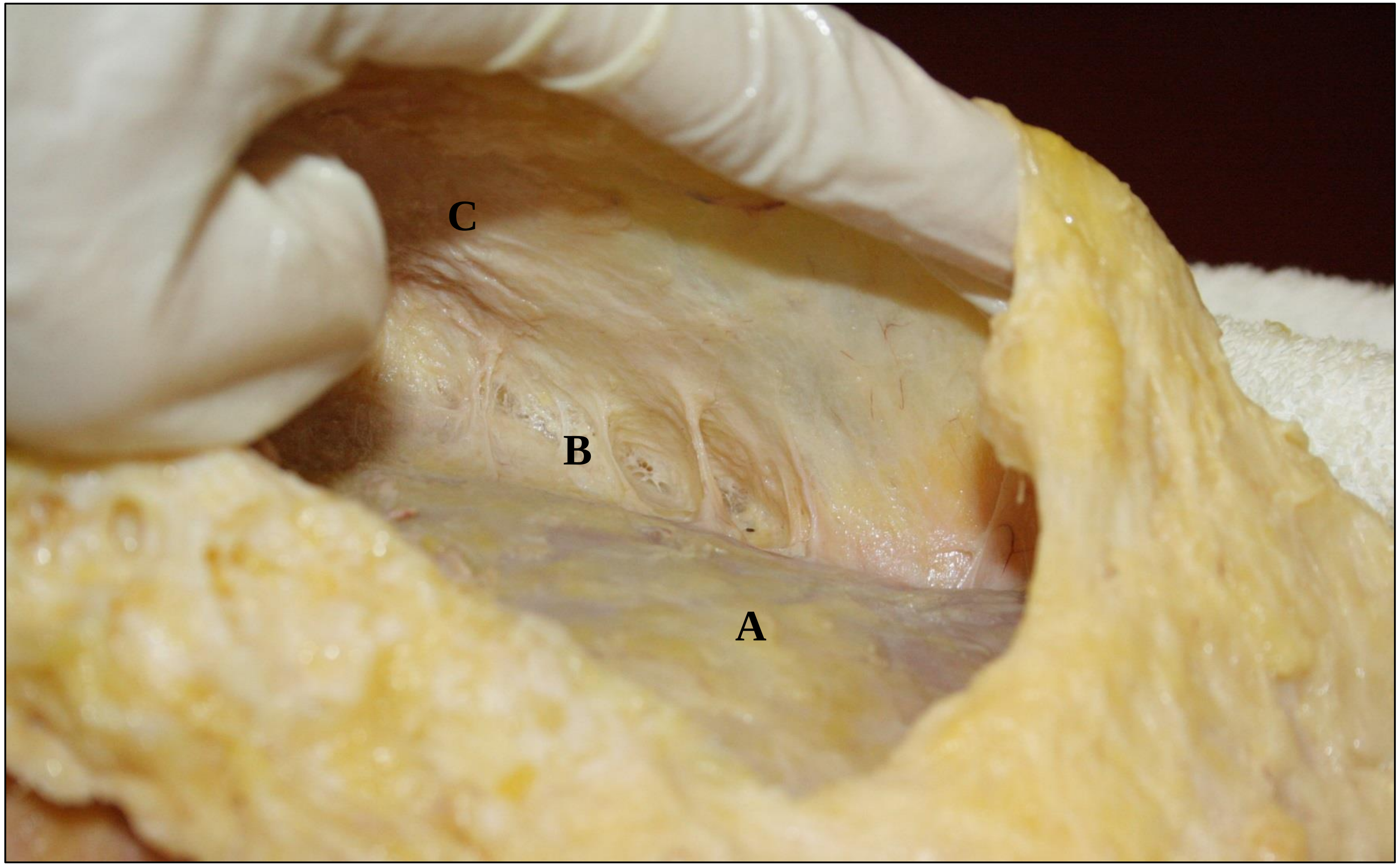

Figure 48: Posterior Extensions of the Posterior Lamellae viewed from the superior aspect. A)Superficial Pectoral fascia, B) Posterior Extensions of the Posterior Lamellae, C) Glandular tissue (reflected).

3.3.3 Muscles within the perimeter

The chest wall muscles that were included within the perimeter of the breasts were the pectoralis major muscle (sternal head), the serratus anterior muscle (anterior fibres), the rectus abdominus muscle (superior fibres) and the external oblique muscle (superior fibres) (Figure 49 and table 10). The sternal head of pectoralis major muscle was found within the perimeter of all of the 12 breasts and made up the largest mean surface area (81 %). The clavicular head of pectoralis major muscle was not found within the perimeter in any of the 12 breasts. It was consistently found to be superior to the superior perimeter of the breast in all 12 breasts. The serratus anterior muscle, rectus abdominus muscle and external oblique muscle were not found within the perimeter of every breast. Serratus anterior muscle was found in nine of the 12 breast perimeters and made up the second largest mean surface area (10 %). External oblique muscle which was only found within five of the 12 breast perimeters, with a mean surface area of 5 % and rectus abdominus muscle which was found in six of the 12 breast perimeters, with a mean surface area of 4 % (Figure 49 and Table 10).

Figure 49: Footprint of the breast with the muscles inside the perimeter shaded. Pectoralis Major muscle (red), Rectus Abdominus muscle (Yellow), Serratus Anterior muscle (Orange), External Oblique muscle (Green).

Chapter 4:

Discussion

This study has contributed to the understanding of the structural anatomy of the breast by quantifying the composition, fibro-adipose structure and attachments of the breast to the chest wall in post-menopausal women. It provides evidence that will enable anatomical and surgical textbooks to develop more accurate and concise written descriptions and anatomical illustrations of the gross structural anatomy of the breast. This study adds to the understanding of the structural anatomy of the breast by providing evidence to base new and more accurate illustrations of the gross anatomical structure of the breast and written descriptions of the ***composition***, ***gross anatomical structure*** and the ***attachments of the breast to the chest wall***. The study found the structural anatomy of the breast to be highly organised and detailed and to be inconsistent with the majority of current anatomical illustrations of the breasts found in anatomical textbooks for undergraduate medicine, surgery and allied health within the major universities across Australia [2-32].

4.1 Composition

4.1.1 Breast Volume and Breast Surface Area

This study is the first anatomical dissection study to quantify the volume (mean 381±272 mL; range: 56-909 mL) and surface area (mean: 302±91 cm^2; range: 187-501 cm^2) of cadaveric breasts using three-dimensional scanning. As such, no such data has been included in any anatomical textbook, despite the fact that *in vivo* data of breast volume and surface area has been collected. The magnitude and range of breast volumes found in the current study in agreement with Hypothesis One were within the range of breast volumes and surface area measurements in *in vivo* studies using three-dimensional scanning, MRI, mammography and water displacement [49, 51-53, 55, 61, 62]. The mean volumes of the breasts in the current study (381±272 mL, range: 56-909 mL) are within the ranges presented in previous invivo studies using three-dimensional scanning (Koch et. al. 2001[62]: 274.4 mL; Coltman et. al. 2017[61]: 680mL; range 75–3100 mL). The lower minimum volume in the current study compared to previous invivo studies may be attributed to the absence of skin in the current study, as well as variations in BMI which is known to affect breast volume (Coltman et al, 2017) (BMI Koch et al. 2011: 22.6 ± 3.3 kg/m², 18-32 kg/m²; Coltman et. al. 2017: 18.4–54.5 kg/m²; Current Study: all cadavers weighed less than 100kg due to the requirement of the UOW Body Donation).

The lower minimum volume measured in the current study compared to previous in vivo studies may also be attributed to the absence of skin in the three-dimensional scan with the breast volume of previous in vivo studies. Multiplying the breast volumes of the current study by the coefficient factor (1.03), as described by Yip et. al. 2012, did little to bridge the gap between the volumes reported in the current study and previous in vivo studies. Quantifying breast volume and surface are in the current study allowed other quantitative data of the structural anatomy of the breast, such as fibro-adipose pocket number to be normalised to breast size.

Although the breast volumes of the current study were within the range of previous *in vivo* studies that utilised measurements methods other than three-dimensional scanning to measure breast volume including water displacement (Vanderweyer and Hertens (2002)[51] mean 446±286 mL, range: 110-1200 mL, n=21 breasts), anthropometric measurements (Huang et. al (2017)[53] mean: 340.0±109.1 mL; range: 91.8–919.2 mL, n=1210 breasts), MRI (Koch et. al. (2011)[62]: mean 457 mL; n=22 and Boyd et. al. (2009) [49]: mean 715.1±496.6 mL; n=100) and mammography (Boyd et. al. (2009)[52] mean 470 mL; range: 63–1564 mL, n=211, mean age: 55 years), the differences in the magnitude of the mean breast volume can be attributed to differences in the measurement methods (MRI taken in prone position, supine position in dissection, standing in anthropometry and the possible inclusion of breast pathologies), as well as cohort number, age and BMI (Vanderweyer and Hertens (2002)[50]: 55 years,: 33-84 years, Huang et. al. (2017) [53]: 49 years, Koch et. al. (2011) [62]: 43 years, Boyd et. al. (2009)[49]: 51 years).

A limitation of this study however was the absence of an anterior chest wall scan data to digitally create the posterior surface of the breast. A recommendation of this study for future cadaveric breast research would be to include anterior chest wall scan data, captured after the final stage of dissection of all of the breasts dissected in the coronal plane to verify the surface of the anterior chest wall. This could then be digitally meshed with the scan of the anterior breast surface in order to create a more accurate 3D digital representation of each coronal breast. This was however beyond the scope of this project as the image processing software did not allow for the combination of multiple scans into a singular 3D object.

The range of surface area measurements (mean: 302±91 cm^2; range: 187-501 cm^2) of the current study were consistent with the range of the previous study found that measured breast surface area in *in vivo* breasts using three-dimensional scanning (Thomson et. al. (2009)[60] mean: 213 cm^2; range: 100-350 cm^2, n=7 females). The volumes and surface area measurements in the current study are the first measurements of cadaveric female breasts with the skin removed. They verify *in vivo* measurements taken with the skin on where the exact boarders of the breast are only estimated beneath the skin.

Quadrant variations

As no previous cadaveric study has investigated breast composition in quadrants of the breasts, the quadrant volume results of the current study can only be compared to the quadrant volume results of one previously published *in vivo* study of 176 women [65]. Although this study also scanned the breasts of these women to determine the volume and divided the scanned breasts into quadrants through the nipple, the position of the body and therefore the breasts during scanning was different (supine in the current study versus standing in the Pandarum et. al. (2011) [65] study). As the breast is a deformable structure, the distribution of the volume throughout the breast may be different in the different positions. This may explain the differences found between the two studies, along with differences in the BMI (current study: all <100kg; Pandarum et. al. (2011)[65] n=83, 23% overweight and (77% obese) and the relative age of the subjects (current study 83 years, range: 63-90 years; Pandarum et. al. (2011)[65] age range: 23-65 years). Both studies found the greatest distribution of the breast volume to be in the superior-medial quadrant, however the quadrant with the least volume was not consistent being the inferior-medial quadrant in the current study while Pandarum et. al. (2011)[65] report it to be the inferior-lateral quadrant. It should be noted that the distribution of breast volume into quadrants described in the current study is specific to post-menopausal cadaveric breasts of elderly women in the supine position. The results however provide quantitative cadaveric data that could be used within anatomical texts. Future anatomical research could investigate a larger cohort, including the breasts of women across a range of ages and BMIs.

4.1.2 Breast Mass

As this is the first anatomical dissection study to quantify the breast mass of cadaveric breasts, the breast mass data cannot be verified to previous dissection mass data. The mean and range of total breast mass measured in the current study (mean: 355±157g; range: 105–598 g) was similar to the breast mass reported in two previous *in vivo* studies which measured the mass of 21 complete mastectomy specimens following pure multifocal in situ carcinomas (Yip et. al. (2012)[86] mean=420±261g, range: 110-1100g; Vanderwyer and Hertens (2002)[51] mean=420±261g, range: 110-1100g). The small difference in the mass reported by Yip et. al. (2012)[86] and the current study can be attributed to the differences in the breast tissue. Yip et. al. (2012)[86] used fresh breast tissue, which may have included the breast pathology, whilst the current study measured embalmed tissue. Previous research (Juang et. al 2011 [90]) suggests Genelyn embalmed cadavers have an increased rigidity in their soft tissues and that the dissection of such tissues is much more difficult to accurately perform when compared to fresh tissues. The limitation measuring the breast mass of embalmed tissue is a disadvantage and as such was only used to investigate the relative percentage mass of the fibro–adipose and fibro-glandular tissue within the embalmed cadaveric breasts. This limitation was confirmed by the lower correlation found between breast mass and breast volume (r^2=0.58). Due to the limitation in the breast mass data, breast volume data was used to normalise most of the quantitative data on the fibro-adipose structure of the breast, such as the number of fibro-adipose pockets, and breast mass was only used to determine the relative percentages of fibro-adipose and fibro-glandular tissue masses of the total breast mass.

4.1.3 Composition of Fibro-adipose and Fibro-glandular tissues

As this is the first cadaveric dissection study to quantify breast composition according to the relative mass of the fibro-adipose and fibro-glandular tissues, the composition data cannot be verified with any previous dissection study, only data from *in vivo* studies. It was hypothesised that the breasts of the cadavers in this study who were all post-menopausal women would contain a higher percentage of fibro-adipose tissue compared to fibro-glandular tissue consistent with previous MRI data of the breasts of post-menopausal women (Boyd et. al. (2009)[49], 71 % mean fibro-adipose tissue, n=100 women, mean age: 49.6 years; Engelken et. al. (2014)[52], 79% mean fibro-adipose tissue (mammography), n=174 women, mean age 55 years, range: 20-79 years). Only two of the 14 breasts examined were in agreement with this hypothesis (both 64 % fibro-adipose tissue), which were two of the largest breasts by volume (959 mL and 746 mL) in the study. The majority of the 14 breasts (n=12) contained a very similar percentage of fibro-adipose (48 %) and fibro-glandular (52 %) tissue. One of the breasts contained a very small percentage of fibro-adipose tissue (28 %) compared to fibro-glandular, interestingly this was one of the smallest breasts by volume (75 mL). The percentage of fibro-adipose tissue composition found in the majority of breasts within the current study were closer to MRI results of younger women (Boyd et. al. (2009)[49] n=400, mean age: 20.8 years), who had a mean of 53% fibro-adipose tissue and the MRI results of women across a wider age range (Lee et. al. (1997)[55] mean 66±18% fibro-adipose tissue, n=40 women, age: 20-83 years). The difference may be attributed to the variation in measurement method (MRI and mammography) versus measurements of cadaveric tissue mass as in the current study. It is also possible that the effect of embalming on the fibro-adipose and fibro-glandular tissue affected their mass differently. Further research could verify this possibility.

The differences may also be due to the individual variation as a wide range of relative percentages of fibro-adipose tissue have previously been found in mastectomy specimens measured using tissue weighing (mean: 14.4 %; range: 7-56 %) and water displacement (mean: 24.9 %; range: 3.6-37.6 %)[51], which have also been found to vary with age and hormonal status. Despite the variation in the mean percentage of fibro-adipose tissue found in the current study (mean: 48 %), the range of percentage mass was consistent with previous research (range: 28-64 %).

The results of the current study provide data on the relative composition fibro-adipose and fibro-glandular tissues by mass of post-menopausal embalmed breasts. This may provide quantitative data on breast composition for anatomical texts. A limitation of the current study was that the percentage composition of fibro-adipose and fibro-glandular tissue within the breasts were determined by mass only and not volume. This was due to difficulties encountered in scanning the dissected fibro-adipose tissues. It is also limited by the use of embalmed tissue, which may impact the breast mass. Consequently, all tissue measurements were taken as relative compositions. Future anatomical studies could overcome this by measuring total volume of the breast and then dissect and scan the fibro-glandular volume to determine the percentage composition by volume. This could then be compared to the data from MRI, mammography and ultrasound studies.

Quadrant Variation

The gland was not found to cover the entire surface area of the breast in any of the dissected breasts, rather some glandular tissue was found in each quadrant. The distribution of the fibro-glandular tissue amongst the quadrants of the breast was in contrast to a systemic literature review on the normal embryology and anatomy of the adolescent female breast[68]. Lemaine and Simmons (2013)[68] reported the superior-lateral quadrant of the breast to have a higher composition percentage of fibro-glandular tissue. Unfortunately their descriptions were not supported by quantitative data as evidence and the study did not report that it specifically measured the distribution of tissues within the breast.

The variation found in the relative percentage of the fibro-adipose and fibro-glandular tissue within the current dissection study and previous *in vivo* studies may assist to explain the discrepancies found in breast composition in anatomical illustrations. The photographic results of the current study provide data on the relative composition of the post-menopausal cadaveric breasts that may be used in anatomical texts and as evidence to base anatomical illustrations upon. It is important that these texts note the agr group that these photographs apply to as *in vivo* studies have found breast composition to change with age. The relative location of fibro-adipose and fibro-glandular tissues within the breast quadrants found in the current study, may assist with the interpretation of MRI, mammography, and ultrasound images of the breast in post-menopausal women and in turn the diagnosis of breast pathologies.

4.2 Gross Anatomical Structure

4.2.1 Fibro-Adipose Structure

Anterior and Posterior Lamellae

In agreement with Hypothesis Three, two fascial sheets were found which were adhered to the anterior and posterior surface of the gland. These results suggest that Cooper's Anterior and Posterior Lamellae and what other studies have referred to as the "anterior and posterior breast capsule" [40, 41, 75] both exist and are the same tissues. The findings are also consistent with the superficial fascial system described by Lockwood (1991) and Scarpa (1809)[40, 75], and more recently by Matousek et. al. (2014)[41]. Previous descriptions of the Anterior and Posterior Lamellae and anterior and posterior breast capsule were limited to the region of the gland, which was known not to cover the entire surface area of the breast. Beyond the borders of the glandular tissue (in all directions), the current study found the Anterior and Posterior Lamellae converge together and continue parallel to each other along the remainder of the breast in a similar manner to the descriptions of the superficial fascial system in the remainder of the body [40, 75]. This is the first time the Anterior and Posterior Lamellae have been explored beyond the glandular tissue.

The anatomical descriptions of the Anterior and Posterior Lamellae of the current study were not consistent with two previous studies however, [42, 45] that described the superficial fascial system as defined by Lockwood (1991) and Scarpa (1809)[40, 75] to lie posterior to a breast capsule. This may be attributed to the dissection method used in these two studies (current study: superficial to deep, Riggio et al (2000)[45]: inferior to superior and Muntan et. al. (2000)[42]: examination of breast slices, stained, embedded in paraffin and sliced again). The majority of the anatomical textbooks used by Australian Universities to teach undergraduate medicine, allied health and science to teach breast anatomy did not include detail of the Lamellae or breast capsule. The most common interpretation of the fascial structure of the breast within the written text and illustrations of most anatomy textbooks was simply that the female breast consisted of five layers; skin (dermis), sub-cutaneous tissue (fibro-adipose), fibro-glandular tissue, retro-mammary space and pectoralis major muscle fascia with Coopers ligaments spanning varying distances between the layers. The results of the current study provide evidence to improve the level of detail on the fibrous tissue within the breast to update anatomical textbooks.

Extensions of the Anterior and Posterior Lamellae

Fibrous tissue was found to span from the entire surface of the Anterior Lamellae to the dermis in agreement with Hypothesis four. This was consistent with Cooper’s Anterior Extensions of the Lamellae Anterior and the “Ligamenta Suspensoria”. This fascial tissue and its location were also consistent with the findings of numerous other studies [36, 39, 42, 45, 74] that unfortunately did not use Cooper’s terminology. These studies either referred to this fascial tissue together with the entire fascia within the breast as “Cooper’s Ligaments” or used inconsistent terminology. The mean length of the Anterior Extensions of the Lamellae Anterior in the current study (measured in 20 ligaments in six breast slices from three breasts, mean 19±5 mm (range: 9-34 mm) was also consistent with the length reported by Cooper (5-25 mm). Cooper however only mentioned this length once throughout his work, in a figure caption[72]. He also provided no detail of the measurement method or the number of cadavers it was measured on. The results of the current study provide evidence for the inclusion of anatomical descriptions of the Anterior Extensions of the Lamellae Anterior or “Ligamenta Suspensoria”, together with quantitative data of their length for accurate and labelled anatomical illustrations.

This study also confirmed the existence of Cooper, Posterior Extensions of the Posterior Lamellae. Consistent with Cooper and Hypothesis five these we found to consist of multiple fibrous bands of tissue running between a layer of loose areola (adipose) tissue from the Posterior Lamellae on the posterior surface of the gland to the superficial fascia of the Pectoralis Major muscle. The current study also extends the descriptions of Cooper by describing the fascial structure beyond the perimeter of the gland, where the same organisation of the tissues was observed. That is, the two fascial layers (Anterior Lamellae and Posterior Lamellae) merged together in the absence of the glandular tissue. At these extremities the Anterior Extensions of the Anterior Lamella and the Posterior Extensions of the Posterior Lamellae were found to run to the dermis and Superficial Pectoral Fascia on either side of this fascial plane. It was not however in the scope of the study to investigate the other fascial tissue of the Lamellae, the Posterior Extensions of the Anterior Lamellae and Anterior Extensions of the Posterior Lamellae as they were not visible macroscopically.

The multiple names and lack of detail in the literature of the fibro-adipose structure explains why the representation of the Coopers Ligaments in anatomical textbooks varies greatly. Most anatomical textbooks provide an ambiguous sagittal diagram of the breast with little to no accompanying text and no quantitative data. This perpetuates the present confusion over the anatomical structure of the fibro-adipose tissue of the breast and therefore there is a particular need to update both the descriptions and illustrations based on the quantitative data collected in this study.

Fibro-Adipose Pockets Anterior and Posterior to the Gland

The fibro-adipose tissue was found to have a compartmentalised design where the adipose tissue was embedded into multiple individual fibrous pockets, which were located both anterior and posterior to the gland. This organisational structure was in agreement with Hypothesis Six and consistent with the descriptions of Cooper and Agur et. al. (1991)[4]. Also in agreement with Hypothesis Eight, a smaller number of fibro-adipose tissue pockets were found anterior to the gland (mean: 35 pockets) compared to posterior to the gland (mean: 53 pockets). The pockets anterior to the gland were observed to be larger (0.89±0.32 mm^2) in size compared to those posterior to the gland (0.33±0.22 mm^2). Although this organisation of the fibro-adipose tissue pocket structure was consistent with Cooper's descriptions, Cooper did not support his descriptions with any quantitative data, nor has any study since, despite published descriptions of this structure.

This study provides for the first time, quantitative data to support these anatomical descriptions. It also provided additional detail on the size, shape and distribution of the fibro-adipose tissue pockets, which has not previously been investigated or published within the literature. Pocket size was found to have a mean adipose tissue mass of 0.4±0.15 g (range: 0.26-0.61 g) and surface area was found to have a mean of 0.88±0.37 cm^2 (range: 0.31-1.97 cm^2). Pocket shape was found to vary over the surface of the breast and to be unevenly distributed across the breast's surface. The shape of the fibro-adipose tissue pockets was more of a square shape with round edges in the superior aspect of the breast and more oval shape in the inferior aspect of the breast and 60% of these pockets were located within the superior half of the breast surface. The detail of pocket number, size, distribution and shape provides important detail for anatomical illustrations of this structure. It should be noted however that the difference found in the pocket shape of the breasts in the different regions of the breasts in the current study may be related to breast ptosis and consequence pocket deformation, which is more likely considering the advanced age of the cadavers involved[61]. Further investigation is required to verify this theory.

The number of the fibro-adipose pockets within the breast and the size of the fibro-adipose pockets were not found to be consistent amongst the breasts of different breast sizes, which was in disagreement with Hypothesis Seven. The mean number of fibro-adipose pockets amongst the 15 breasts dissected was 200 pockets (range: 108-306), with a positive correlation was found between the number of fibro-adipose tissue pockets and breast volume, as well as breast surface area. This finding suggests that as breast size increased (by volume and surface area), so did the number of fibro-adipose tissue pockets. However, when the number of fibro-adipose pockets within the breasts of the upper and lower tertiles by volume were compared, they were not statistically significantly different, with only a trend for fibro-adipose pocket number to be greater in breasts of a higher breast volume. Further research is required to verify whether breasts of a larger size have a greater number of fibro-adipose pockets.

The very weak correlations between fibro-adipose pocket size (mass and surface area) and breast volume and the lack of significant difference found between pocket mass/surface areas of the breasts of the upper tertile by volume compared to the lower tertile by volume suggest that larger breasts do not have fibro-adipose pockets that are larger in size. The results of the current study suggest that it is more likely that breast size increases by increasing the number of fibro-adipose pocket number rather than by increasing the size of fibro-adipose pockets. again further research is required to verify this notion

This data on fibro-adipose pocket number, size, shape, distribution and how these vary with breast size provides evidence from which accurate anatomical illustrations and written anatomical descriptions can be based. Many illustrations within current anatomical texts display no discernible organisation of fibro-adipose tissue and limit descriptions of this complete structure to fibrous septa separating the glandular tissue extending from the dermis to the superficial pectoral fascia [4-6, 9, 17, 20, 26, 28]. Many sagittal illustrations of the breast also neglect to include the fibro-adipose tissue located posterior to the gland. There is an urgent need for an update on the detail of the breasts structural anatomy currently being taught from these textbooks

4.2.2 Location of the Fibro-Glandular Structure

The current study was limited in its investigation of the fibro-glandular structure to its regional location within the breast and its relative contribution to breast composition (see 4.1). Consistent with Cooper[1], the fibro-glandular tissue with the body in a supine position was found to be predominantly located in the inferior half of the breast. The location was inconsistent however with two other studies (one dissection and one *in vivo* study) [38, 44] and many anatomical textbooks that found or reported the glandular tissue to be predominantly in the superior-lateral quadrant projecting into the axilla as the Tail of Spence. These studies had extensive descriptions of the micro-anatomy of the breast but very little detail of the methodology of how the location and distribution of the fibro-glandular tissue within the breast was measured or where they sourced such information. The differences may be attributed to differences in the position of the breasts in the current study during dissection. The breasts measured in the coronal plane were dissected in the supine position. This was done as the chest plate was still intact and as such the position of the torso, in supine, was necessary for dissection as the weight was too great to suspend in any other position. The tissues of the embalmed cadaveric breast are however deformable and this may have attributed to the differences in the measurements of the current study to previous studies, as some of the tissue may have changed relative locations. However, the positions of the breasts in current study were the same as those reported by Cooper (1840) and as such a comparison could be made between the outcome measures of the two studies. Also the relative age of the cadavers or women investigated in *in vivo* studies may affect the relative size and location of the gland in the breast. Indeed many textbooks depict an illustration and description of nulliparous or primiparous breasts, whereas the current study represented the post-menopausal breast, with the mean age of the cadavers investigated in the current study being 82 years.

The data collected in the current study on the gross anatomical structure of the fibro-adipose and fibro-glandular tissues and their distributions within the breast provides the quantitative data to base illustrations of the gross anatomical structure of the breast for future anatomical texts based on the post-menopausal cadaveric breast in the supine position. Increased understanding of the complex fascial three-dimensional cobweb that encases the adipose tissue within the breast may also assist to explain the scarring that can occur within the breast following breast surgery, such as a lumpectomy. It also provides useful information about the basic design of breast tissue for three-dimensional printing of the breast and for surgical breast reconstruction.

4.3 Attachment of the breast to the chest wall

Footprint of the breast

The regional anatomy of the breast in terms of its location on the chest wall was consistent with Cooper[1] and previous anatomical studies that described the breast to sit between the 2-6/7th ribs and extend from the lateral edge of the sternum to the mid-axillary line. The current study added to the body of knowledge by providing quantitative data from the number and age of the cadavers this regional anatomy description is based on. The consensus in the literature regarding the location of the breast on the chest wall may explain why this detail is commonly included in anatomical texts.

The length of the perimeter of the cadaveric breasts found in the current study (mean: 46.01±12.2 cm; range: 33-58 cm) cannot be compared to previous research as it has not previously been measured. The length was within the range however of previous studies that have measured sections of the perimeter, namely the inframammary fold (10-12 cm in length[77, 80]), the diameter of the breast (14.3±1.4 cm in length, range: 8.5–23.5 cm[53]) and the lateral perimeter (7-13 cm in length[36]).

Consistent with previous dissection studies[34-37, 40-43, 45, 48], descriptions in anatomical textbooks [2, 4, 6, 7, 9-14, 19, 21-23, 27] and in agreement with Hypothesis 10, the muscles found within the perimeter the breast were Pectoralis Major, Serratus Anterior, Rectus Abdominus and the External Oblique muscles. This is also consistent with Cooper, except his descriptions did not include the Rectus Abdominus muscle. This study is also consistent with two studies[43, 78] that described the inframammary fold (inferior perimeter attachment) to consistently sit below the inferior margin of the Pectoralis Major muscle and the Pectoralis Major contributed the largest surface area (two thirds) to the footprint of the breast. Unfortunately, no quantitative data was provided to support these descriptions. The current study is consequently the first study to provide quantitative data on the relative contribution of each muscle to the footprint of the breast. Consistent with previous studies and anatomical texts, the Pectoralis Major muscle was present within all breast perimeters and was found to contribute the largest surface area (81%) of the footprint of the breast. The footprint of the breast varied however in the mean contributions of the Serratus Anterior muscle (10%), which was found within nine of the 12 breast perimeters, External Oblique muscle (5%), which was found within five of the 12 breast perimeters and Rectus Abdominus muscle (4%), which was found within six of the 12 breast perimeters.

The data provides evidence for anatomical textbooks about the muscles adjacent to the posterior wall of the breast. It also provides evidence of the shape of the posterior wall of the breast, based on the adjacent muscle architecture, which has significant application in three-dimensional digital scanning and digital breast reconstruction. Current three-dimensional breast reconstruction creates a three-dimensional breast created with a posterior wall, which has a curved shape [60, 61]. This study provides new evidence of the true shape of the posterior surface of the breast for digital breast reconstruction. It may also assist breast surgery rehabilitation of the muscles that could be affected by surgery such as mastectomy.

4.3.1 Posterior and Perimeter Attachments

Posterior Attachments

In agreement with Hypothesis Five and Nine, the posterior surface of the breast was found to attach to the superficial pectoral fascia via numerous but weak fibrous bands that house small adipose pockets in a similar fashion to the superficial fibro-adipose structure namely Cooper's Posterior Extensions of the Posterior Lamellae. This is consistent with previous studies that describe small fibres within the "retro-mammary space" or "bursa" (containing small areolar tissue) that allow the breast to "glide freely" on the chest wall [37, 45, 66, 68, 70, 78, 80, 88] .

Consistent with Wűringer et. al. (1998) [48] (cadaveric study of 28 cadaveric breasts, age range 68-92 years), a horizontal septum running from the nipple to the posterior wall of the breast which divided the breast into a superior two thirds and inferior one third was consistently found in both the coronal and sagittal dissections of the current study. Contrary to the findings of Wűringer et. al. (1998) [48] however, the attachment of the horizontal septum to the chest wall was not considered to be strong as it was required only blunt dissection, similar to the other posterior breast wall attachments. Also in contrast to the findings of Wűringer et. al. (1998) [48], no periosteal attachment of the horizontal septum was found. The difference may be attributed to differences in the direction and planes of dissection (cranial to caudal direction - transverse plane (Wűringer et. al. (1998) [48] study) and coronal and sagittal plane (current study)). Although the sagittal plane dissection in the current study clearly visualised both attachment ends of the septum perhaps with greater clarity compared to the transverse plane dissections Wűringer et. al. (1998) [48], it is also possible that due to the plane of the sagittal breast slices, that a single rib periosteal attachment was missed. Further research is required that dissects the breast in multiple planes to verify whether there is any periosteal attachment. Irrespective of any periosteal attachments, the results of the current study do question however the strength of the attachment of the horizontal septum as it required only blunt dissection to separate it from the anterior chest wall in all of the dissections of the current study.

The horizontal attachment observed in the current study was different to the description of the Triangle Fascial Condensation observed by Matousek et. al. (2014)[41]. Matousek et. al. (2014)[41] described a fascial system that spanned from the inferior portion of the breast to the chest wall, inserting on to the 5th rib, however no periosteal attachment was observed in the current study. No photographic evidence was provided of this by Matousek et. al. (2014)[41], only an illustration of a mid-sagittal section of a pert shaped breast with a fascial system inserting on to the 5th rib. It is possible however that a fascial attachment to the 5th rib was not identified in the current study due to the plane of the sagittal breast dissection. Due to the age of the cadavers and the supine position that they were embalmed, the breasts were commonly splayed to the side of the anterior chest wall and not pert in their shape. Consequently, a mid-sagittal section of the breast did not cut through the ribs in a sagittal plane, but rather an oblique plane. This oblique angle may have obscured the view of a fascial attachment to the 5th rib. The current study also consistently found adipose and glandular tissue in the inferior aspect of the breast in the region Matousek et. al. (2014)[41] described as the Triangle Fascial Condensation. Although Matousek et. al. (2014)[41] stated that this region consisted of dense fascial tissue with no breast tissue, in their images of the sagittal slices of the cadaveric breast, breast tissue can clearly be seen within this area. This is inconsistent with the current study which not only observed breast tissues in this region but was able to both weight the tissue and measured the tissue pockets dimensions. The observed difference may be attributed to the different dissection methods used. The classical techniques used in the current study did not compromise the facial tissue compared to the chemical dissection techniques used by Matousek et. al. (2014)[41] . In agreement with Matousek et. al. (2014)[41] however, the current study considered the perimeter attachments to be stronger than the posterior wall attachments as they required sharp dissection along the entire perimeter..

Perimeter Attachments

The breast was found to be attached to the anterior aspect of the chest wall along its entire circumferential perimeter. The attachments of the medial and lateral aspects of the **superior arc attachment** were found to anchor to the superficial pectoralis major muscle fascia consistent with the findings of Jinde et. al. (2006)[37]. In contrast to Jinde et. al. (2006)[37] however, the middle (most superior) section of the superior arc perimeter attachment was consistently found in all of the coronal dissections to dive between the sternal and clavicular heads of the pectoralis major muscle and intersect with the deep pectoralis muscle fascia. As both the superficial and deep pectoralis major muscle fascia attach to the clavicle, the results of the current study were also consistent with the findings of Matousek et. al. (2014)[41], who reported that superior attachment of the breast attaches to the clavicle. This clavicular attachment however was described by Matousek et. al. (2014)[41] to be via superficial and deep clavicular ligament whereas the current study found the connection to be via the superficial and deep pectoralis muscle fascia. Irrespective of this difference in terminology, both studies found an indirect and distant connection between the superior arc perimeter attachment of the breast and the clavicle (via muscle fascia or ligaments to the clavicle), with a considerable distance of fascial tissue between the attachment. This extensive fascial connection may assist to explain the ptosis of the breast with age.

The current study found the **medial arc attachment** of the breast required the most time to dissect sharply in comparison to the other regions of the perimeter attachments. The periosteal attachment to the lateral aspect of the sternum was consistently found to be composed of dense collagen fibres with very little adipose tissue. These findings were consistent with the descriptions of the medial perimeter attachments in previous cadaveric dissection studies[36, 37, 41, 78, 81] (including Cooper) which describe the medial attachment to consist of short, strong fibres running in a horizontal direction from the dermis to the periosteum, which required sharp dissection. The medial arc attachment of the perimeter attachment observed in the current study was contradictory to the descriptions of Wűringer et. al. (1998)[48] who described the medial attachment as a weak attachment, which allowed the breast to deform laterally. Wűringer et. al. (1998)[48] description however was not supported by any quantitative data. The relative "strength" of the medial aspect of the perimeter attachment may explain why the medial boarder of the breast can be consistently visualised in the post-menopausal breast and why disruption of the medial tissues during breast surgery can lead to poor aesthetic results, particularly in breast augmentations[67]. The results also emphasise the importance of the medial attachment for breast aesthetics during breast surgery.

The **lateral arc attachment** was found to be consistent with previous research [36, 37, 41, 48] which described it to traverse the lateral aspect of the pectoralis major muscle and "loosely" attach the breast to the axillary chest wall [36, 37, 48, 81]. The inferior aspect of the lateral arc attachment was found to be a sling of fascia formed by the merging of muscle fascial sheets arising from the edges of the serratus anterior muscle and the external oblique muscles (Figure 47). The inferior portion of the attachment was similar to that described by Matousek et. al. (2014)[41], however the current study has provided much more detail on this structure. The current study investigated this region in both the sagittal and coronal planes using classical dissection techniques whereas the Matousek et. al. (2014)[41] used chemical dissection to reveal the structures. Pilot testing in the current study revealed the connective tissues of the breast were affected by chemical dissection and as such were not consistently reliable dissection techniques. As such, the current study has been able to illustrate clearly, through cadaveric images, the organisation of the attachment of the breast to the chest wall in both the sagittal and coronal aspects whereas the Matousek et. al. (2014)[41] study only represented this attachment as a line drawn on an image of a female breast with the skin on. No other studies have described or illustrated the fascial arrangement of the inferior-lateral portion of the perimeter attachment.

The **inferior arc attachment** of the breast, also known as the Inframammary Fold (IMF) was found to be a tightly adhered band of tissue anchoring the inferior portion of the breast strongly to the muscle fascia's of the anterior chest wall. The relative location of the inferior perimeter attachments as described in previous studies is consistent with the current study however, the attachment site (periosteal or fascial) was unable to be confirmed in the current study as in most instances the pectoralis major muscle, or the angle of the bandsaw slice obscured the attachment. The inferior attachment in the coronal plane dissection of the current study was seen to attach to the muscle fascia of the anterior chest wall muscles, deep to which it could not be observed to attach to the periosteum of the ribs. This is consistent with other research that has also described the IMF (inferior perimeter) attach solely to the muscle fascia[81, 82]. As the IMF in the current study required sharp dissection to be removed from the anterior chest wall during the coronal dissection, it suggests there could be a periosteal attachment. This connection however was not clearly visible on the coronal dissections which may explain why previous studies may not have found this connection. It was however beyond the scope of this study to investigate.

The results of the sagittal dissection in the current study could not verify the results of previous studies that have described a periosteal attachment of the inframammary fold[34, 36, 37, 41, 48] or a pure muscle fascia attachment [81, 82]. The periosteal attachments of the inframammary fold have been reported to be located at the 5th rib [40, 41, 48] and 6th ribs [34]. Consistent with Matousek et al. (2014)[41] the 5th rib was exposed to breast tissue below the inferior border of the pectoralis major muscle and above the superior border of the rectus abdominus muscle and external oblique muscle, which could indicate a location of a fibrous attachment from the breast to the chest wall. However, this was not clearly visible as the slices were taken through fixed points on the breast, and due to the deformation and displacement of the embalmed breasts, these were not true sagittal slices through the ribs. Consequently some slices were unable to demonstrate successfully the entire breast depth from the skin to the ribs, and as such the deep attachment of the IMF could not be observed.

The current study was unable therefore to verify the existence of the triangle fascial condensation attaching to the 5th rib as described by Matousek et. al. (2014)[41], this is likewise perhaps due to the angle of the cut through the breast in the sagittal plane rather than through the midline of the breast. The current study did however demonstrate the presence of breast tissue in the inferior portion of the breast which was inconsistent with the findings of Matousek et. al. (2014)[41] which described only fascial attachments of the dermis to the 5th rib in this region. The difference may be attributed to the chemical dissection techniques used by Matousek et. al. (2014)[41], which would have removed any adipose tissues from this region, whereas the sagittal slices in the current study did not remove any adipose tissue. Further research could include a dissection technique that clearly shows, by use of bandsaw slicing, the perpendicular aspect of the breast rather than a classical sagittal aspect in order to verify the presence of any periosteal attachment of the inframammary fold (inferior perimeter) to the chest wall.

4.4 Conclusion

This study adds to the understanding of the structural anatomy of the breast in terms of the ***composition of the breast*** by providing data on the relative percentage of the fibro-adipose and fibro-glandular tissues by weight; ***gross anatomical structure of the breast*** by providing quantitative data on the number, size, shape and distribution of fibro-adipose pockets in both the coronal and sagittal plane, and quantitative data on the length of the "Ligamenta Suspensoria". It also provides some consistency in the naming of the fascial tissues that form the ***fibro-adipose structure***. Lastly it provides knowledge on the relative strength and location of the ***posterior and perimeter attachments*** of the breast to the chest wall and quantitative data of the muscles that form the footprint of the breast on the anterior chest wall.

This data as a whole provides evidence to base new and more accurate illustrations of the gross anatomical structure of the breast and written descriptions of the ***composition***, ***fibro-adipose structure*** and the ***posterior and perimeter attachments of the breast to the chest wall***, which are currently lacking in anatomical texts used to teach this breast anatomy to medical, allied health and science undergraduate students within Australian Universities. Greater understanding of the structural anatomy of the breast may assist clinically in the diagnosis of breast pathologies and the aesthetic outcomes of breast surgery. It may also assist in the design of the breast tissue from three-dimensional printing or in the reconstruction of breast tissue.

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Appendices

Appendix A: Textbook review

A.1 Review of the descriptions and illustrations of breast anatomy presented in anatomical textbooks for Medicine and Allied Health subjects in Universities across Australia

Textbook	Total no. Images	No. Sagittal Images	Sagittal Image/s Showing	No. Coronal Images	Coronal Image/s Showing	Fibro-adipose structure	Fibro-glandular structure	Composi -tion	Peri -meter	Data
Abrahams et al. 2005	3	1 anterio-medial	1 surface 1 photo	2	1 photo, 1 regional	C	L	Co	RM	
Abrahams et al. 2008	5	2	1cadaver 1structure	3	2 photo 1surface 1 cadaver 1 structure					
Agur and Dalley 1991	3	1	structure	2	1 regional 1 structure	CP	LS		R	
Applegate 2000	2	1 anterio-medial	structure	1	surface	C	LS		M	
Clemente 2007	9	3	1 structure 1 regional 1 surface	6	2 photo 5 surface 1 regional	C	LS		RM	
Drake et al. 2005	1	0		1	regional	C	GL	Co	RM	
Eizenberg et al. 2008	0	0		0				Co		
Ellis 2002	1	0		1	regional		LS		RM	

C=Coopers Ligament, P=pocket structure, G=modified sweat gland, L=15-20 lobules, S=septa/stroma, Co=composition, A=attachment to wall, M=muscles within, R=regional anatomy

Textbook ctnd.	Total no. Images	No. Sagittal Images	Sagittal Image Showing	No. Coronal Images	Coronal Image Showing	Fibro-adipose structure	Fibro-glandular structure	Composi -tion	Peri -meter	Data
Gilroy 2013	2	1	structure	1	surface	C	S	Co	RM	
Gilroy et al. 2012	5	1	structure	4	2 surface 2 structure	C			RM	
Gosling et al. 2003	2	2	1 cadaver 1 structure	0			LS		RM	
Hansen 2014	3	1	structure	2	1 structure 1 surface	C	GL		R	
Hansen and Lambert 2008	5 + 17 clinical	2	structure	3	1 structure 1 regional 1 surface		G		R	
Kapit and Elson 1993	2	1	structure	1	regional	C	L	Co		
Lindsay 1995	2	1	structure	1	structure	CP	L			
Marieb and Hoehn 2012	2	2 1 anterio-medial	structure	0			GLS			
McKinley and O'Loughlin 2006	1	0		1	surface photo					
McMinn 1991	0	0		0		CP	GL		RM	
Moffat 1993	1	0		1	surface		LS		M	

C=Coopers Ligament, P=pocket structure, G=modified sweat gland, L=15-20 lobules, S=septa/stroma, Co=composition, A=attachment to wall, M=muscles within, R=regional anatomy

Textbook ctnd.	Total no. Images	No. Sagittal Images	Sagittal Image Showing	No. Coronal Images	Coronal Image Showing	Fibro-adipose structure	Fibro-glandular structure	Composi-tion	Peri-meter	Data
Moore et al. 2015	5 + 6 clinical	1 anterio-medial	structure	4	structure	C	GL		RM	
Moore et al. 2014	6	1 anterio-medial	structure	5	2 regional 3 structure	CP	GL		RM	
Moses et al. 2005	6	1	cadaver	5	1 cadaver 4 structure		G		R	
Olson 1996	1	0		1	regional					
Rohen et al. 2016	4	1	1 cadaver 1 structure	3	2 cadaver 1 structure 1 regional					
Saladin 2007	3 + 2 clinical	2	1 cadaver 1 structure	1	structure	C	GLS	Co	M	
Sinnatamby 2006	0	0		0		C	GL		RM	
Snell 1995	4	2		2		C	LS		RM	
Tank and Gest 2008	4	1	structure	3	structure					
Tortora and Derrickson 2012	2	1	structure	1	structure	C	GL		M	
Tortora et al. 2015	2	1	structure	1	structure	C	GL		M	
Van De Graff et al. 2002	3	1	structure	2	1 regional 1 structure	C	L		RM	

C=Coopers Ligament, P=pocket structure, G=modified sweat gland, L=15-20 lobules, S=septa/stroma, Co=composition, A=attachment to wall, M=muscles within, R=regional anatomy

Appendix B: Written descriptions for anatomical textbooks

B.1 Breast Composition

Dissection research has reported breast volumes to range from a mean of 381±272 mL (range: 56-909 mL) (Gaskin et al. 2017) while *in vivo* studies using three-dimensional scanning, MRI, mammography and ultrasound have reported larger mean values with breast volumes ranging from 75 to 3100 mL [49-53, 55, 60-62, 89]. Breast volume has been shown to vary with age, BMI and hormonal status[51, 61].

The female breast is comprised of fibrous, adipose and glandular tissues of varying relative percentages. The relative contributions of each of the tissues vary with age, BMI, hormonal status and measurement methods. *In vivo* studies have found the young nulliparous breast to have an essentially even contribution by volume of the fibro-adipose and fibro-glandular tissue[49], whereas the multiparous or post-menopausal breast has been reported to have a higher percentage of adipose tissue by volume[49, 52]. The fibro-adipose and fibro-glandular composition of the breast, based on mass of the tissues in relation to total breast mass, has been investigated in a dissection study to have comparable contributions (48% fibro-adipose tissue, 52% fibro-glandular tissue) hence highlighting the difference in relative composition based on measurement methods[51, 55].

B.2 Fibro-Adipose Structure

The fibro tissue of the breast is organised into a highly detailed and complex three-dimensional structure. There are two fascial layers seen throughout the entire breast, the Anterior (superficial, Figure A) and Posterior (deep, Figure B) Lamellae located anterior and posterior to the gland. Beyond the perimeter of the gland, these two fascial layers merge together to form a dual layer of fascial tissue. Dissection research has shown both Lamellae to each have a set of Anterior and Posterior Extensions (Figure C) dispersing from them in an anterior and posterior direction respectively which form a three-dimensional 'cobweb' within which the glandular and adipose tissues are encased (Figure D). Throughout the breast, in both the presence and absence of the glandular tissue, the Anterior Extensions of the Anterior Lamellae run from the Anterior Lamellae to the dermis. These are what Cooper referred to as "Ligamenta Suspensoria". The Posterior Extensions of the Posterior Lamellae likewise span from the Posterior Lamellae to the superficial fascia of the Pectoralis Major muscle. The Posterior Extensions of the Anterior Lamellae and the Anterior Extensions of the Posterior Lamellae are unable to be differentiated in the presence of glandular tissue, however beyond the perimeter of the glandular tissue of the breast, they can be observed to span from their respective Lamellae to merge with each other. Beyond the perimeter of the breast the same organisation is observed but it is known as the "Superficial Fascial System" as described by Lockwood (1991) [40], or "Scarpa's fascia" as described by Scarpa (1809) [75]."

Figure A: Anterior Lamellae viewed from lateral aspect, (★) borders of the breast highlighted by viscous dye injections.

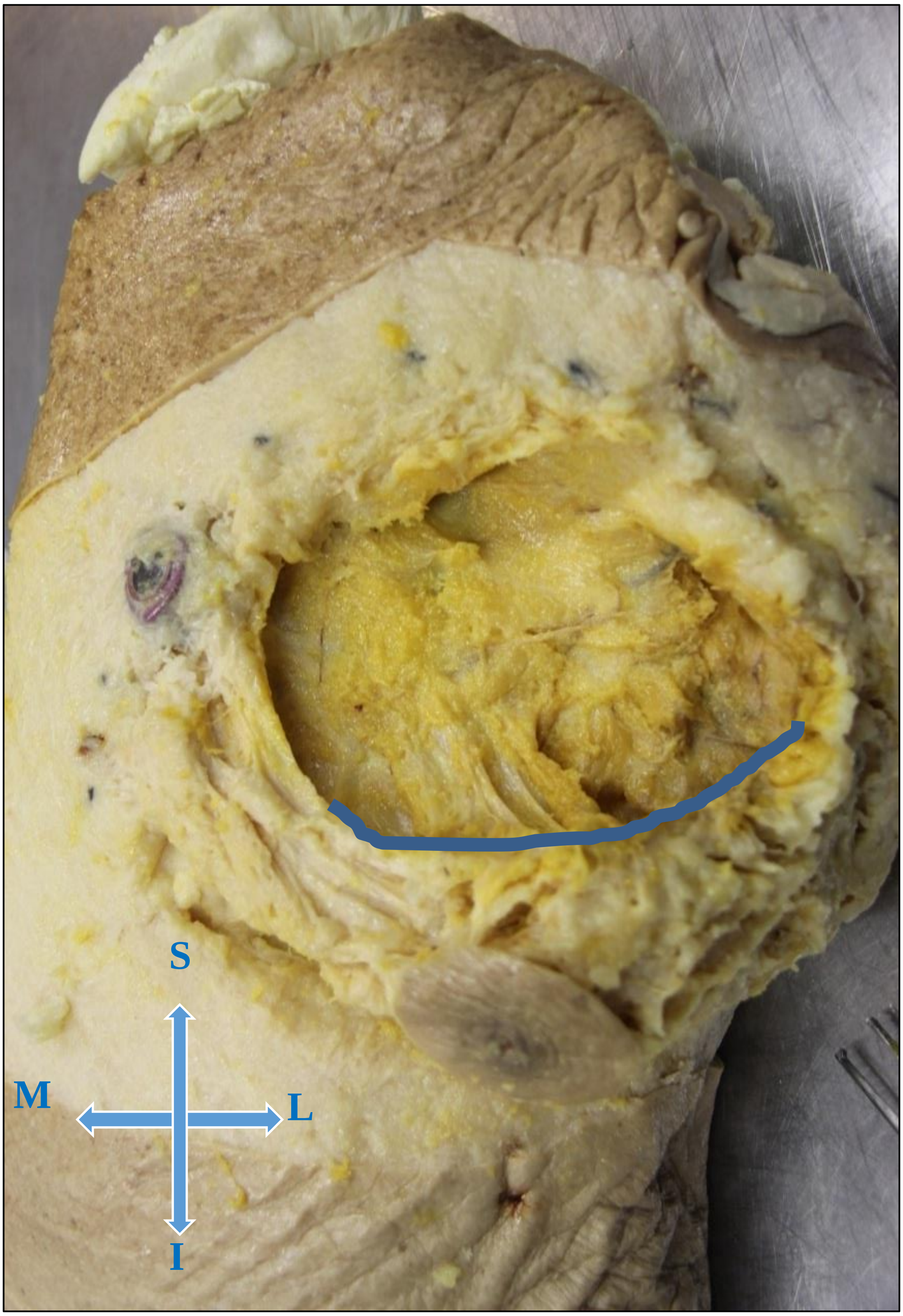

Figure B: Coronal dissection of the breast showing the fibro-adipose tissue deep to the fibro-glandular tissue and the Posterior Lamellae (reflected and coloured dark blue).

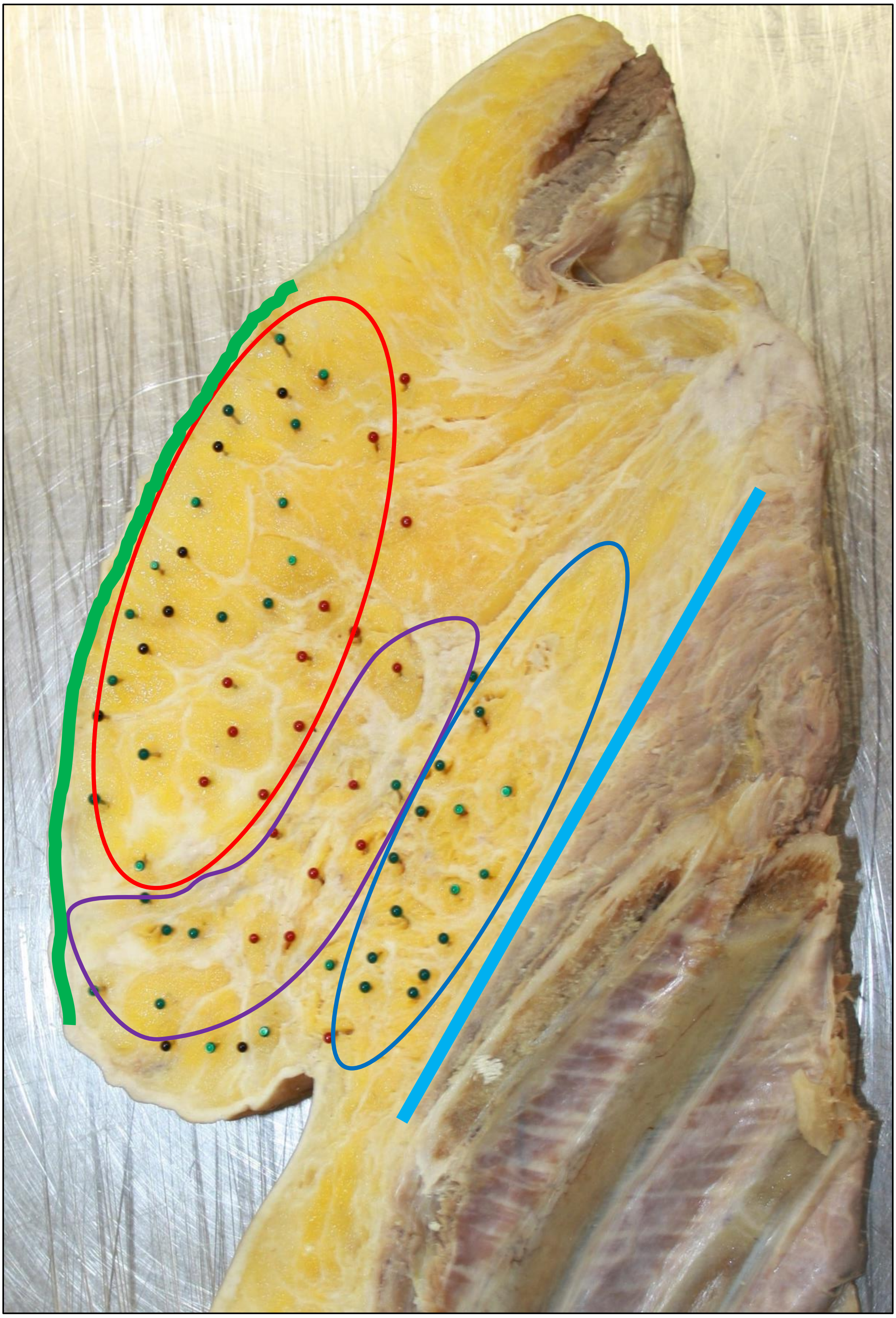

Figure C: Mid-sagittal slice of the breast through the nipple showing the dermis (green) and the Superficial Pectoral fascia (light blue). The regional depths are circled anterior to the gland (red), within the gland (purple) and posterior to the gland (dark blue).

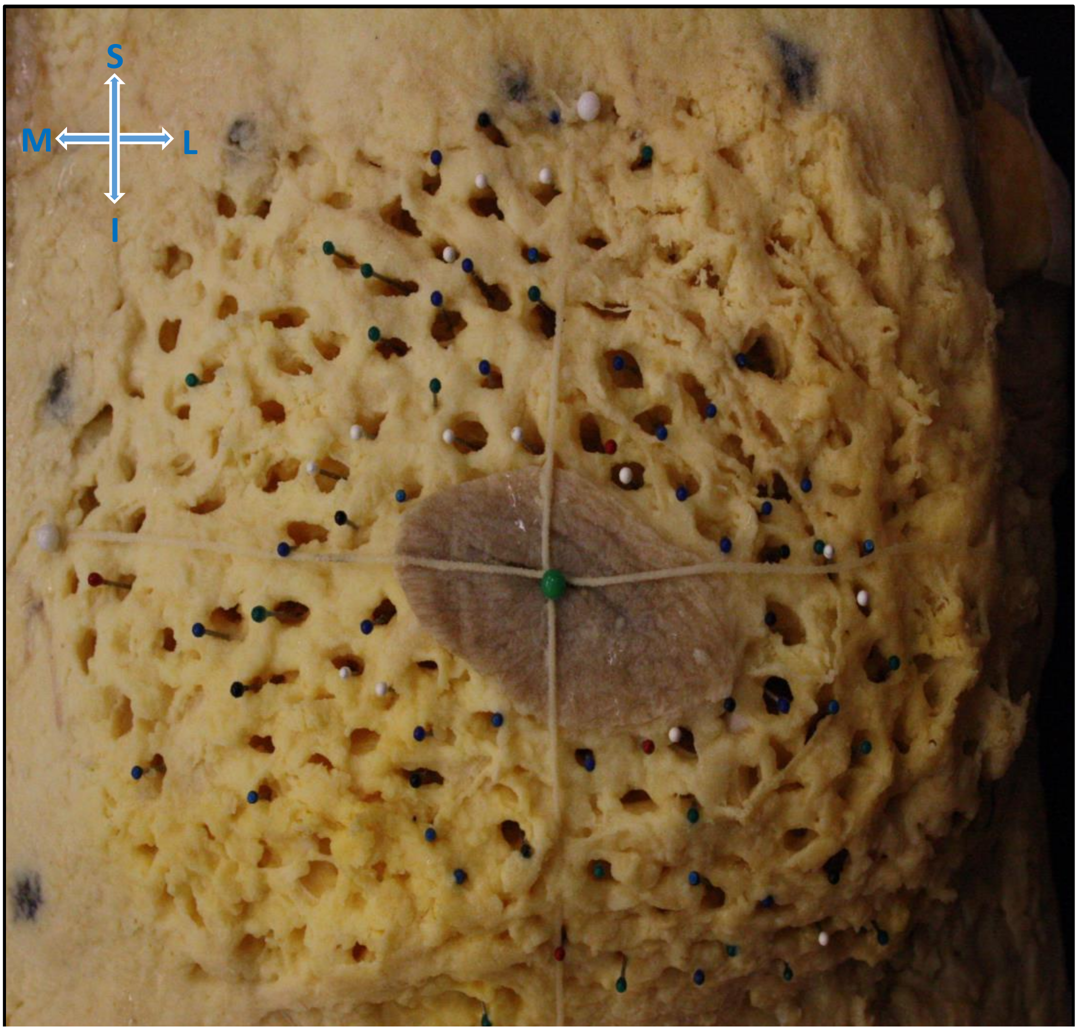

Figure D: Example of a coronal dissection of the breast showing the adipose tissue removed and pockets pinned for further quantitative analysis.

B.3 Attachment of the Breast to the Chest Wall

The female breast sits on the anterior chest wall and spans between ribs two to six in the vertical plane and between the mid-axillary line and the lateral sternum in the horizontal plane. The breast is attached firmly to the chest wall via posterior and perimeter attachments (Figure F and Figure G). The posterior attachments, named by Cooper to be the Posterior Extensions of the Posterior Lamellae, run from the Posterior Lamellae of the breast to the Superficial Pectoral Fascia (Figure F). These are numerous spanning across the entire surface area of the posterior aspect of the breast and weak compared to perimeter attachment as they only require blunt dissection and have no periosteal attachment. The perimeter attachment has both fascial and periosteal attachments along its length. It can be considered to consist of four arcing components, the superior, medial, lateral and inferior attachments (Figure G). The relative strength of these components of the perimeter is controversial with some authors suggesting the medial attachment is the strongest, whereas other texts describe the inferior attachment to be the strongest consistently. Direct periosteal attachments are considered to be stronger then fascial attachments and both the inferior perimeter (to the 4^{th} or 5^{th} rib) and the medial perimeter (to the lateral edge of the sternum) have been found to have a periosteal attachment. The superior attachment of the breast penetrated deep between the two heads of the Pectoralis Major muscle and attaches to the Deep Pectoral Fascia (Figure G). The lateral breast perimeter is attached to the fascia of the superficial chest wall muscles. The muscle fascia’s of Pectoralis Major, Serratus Anterior, External Oblique and Rectus Abdominus muscles merge at the inferior-lateral aspect of the breast perimeter and continue as a combined structure superiorly towards the axilla (Figure G). The lack of periosteal attachments of the superior and lateral border may contribute to breast ptosis with age.

The length of the perimeter of the breasts found in a dissection study to be 46.01±12.2 cm (range: 33-58 cm) Previous *in-vivo* studies have measured part of the perimeter length, particularly the inframammary fold (10-12 cm in length[77, 80]), the diameter of the breast (14.3±1.4 cm in length, range: 8.5–23.5 cm[53]) and the lateral perimeter (7-13 cm in length[36]), rather than the entire perimeter. The muscles within the perimeter of the breast, and therefore having a direct relationship with the footprint of the breast are the Pectoralis Major, Serratus Anterior, External Oblique and Rectus Abdominus muscles (Figure H). A dissection study (n=12) revealed the Pectoralis Major muscle was present within all breast perimeters and was found to contribute the largest surface area (81%) of the footprint of the breast. The footprint of the breast varied however in the mean contributions of the remaining three muscles. The Serratus Anterior muscle, which was found within 75% of breast perimeters, was found to contribute 10% of the surface area of the breast footprint, the External Oblique muscle, which was found within 42% of breast perimeters was found to contribute 5% and Rectus Abdominus muscle, which was found within 50% of breast perimeters was found to contribute 4% (Figure H).

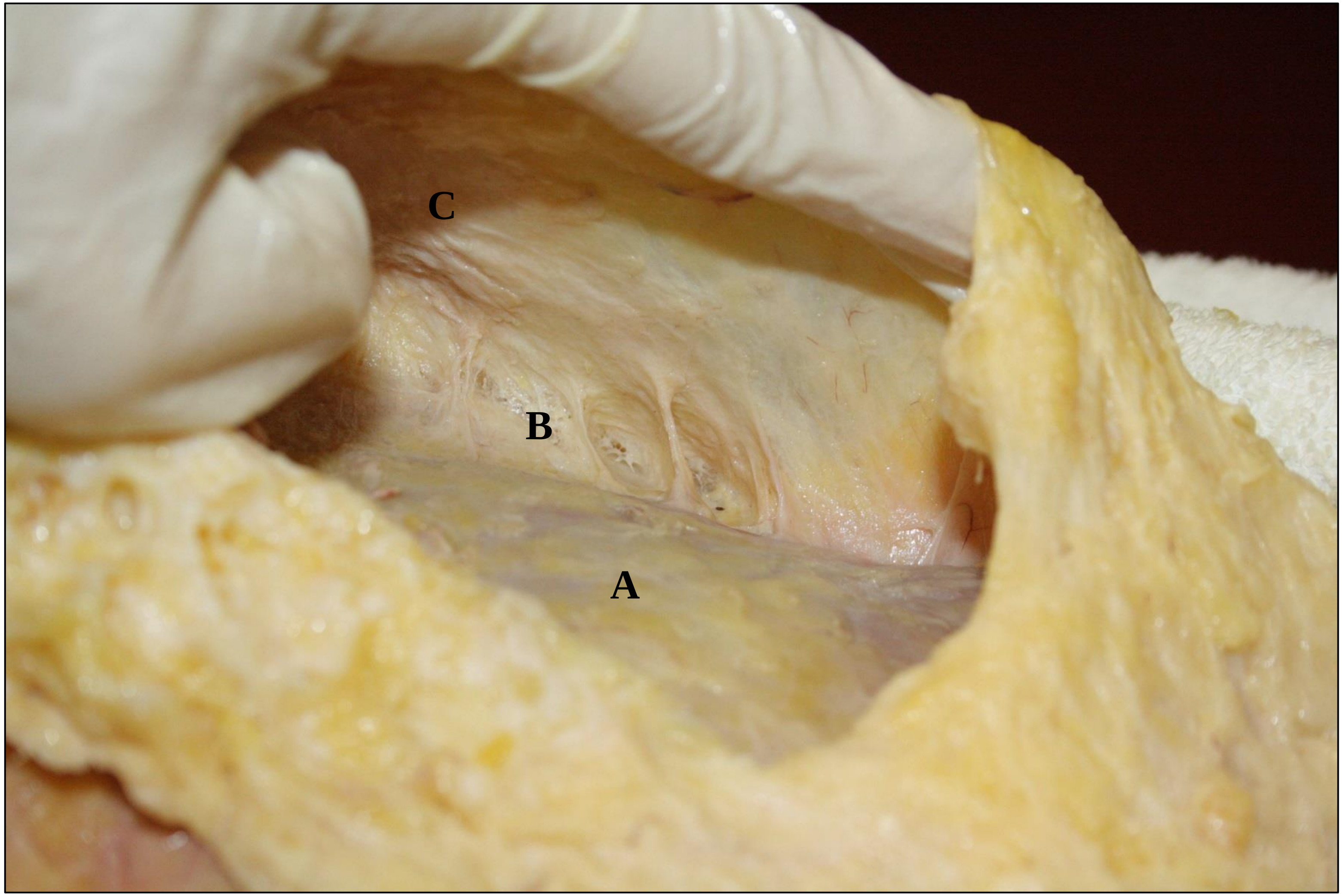

Figure F: Posterior Extensions of the Posterior Lamellae viewed from the superior aspect. A)Superficial Pectoral fascia, B) Posterior Extensions of the Posterior Lamellae, C) Glandular tissue (reflected).

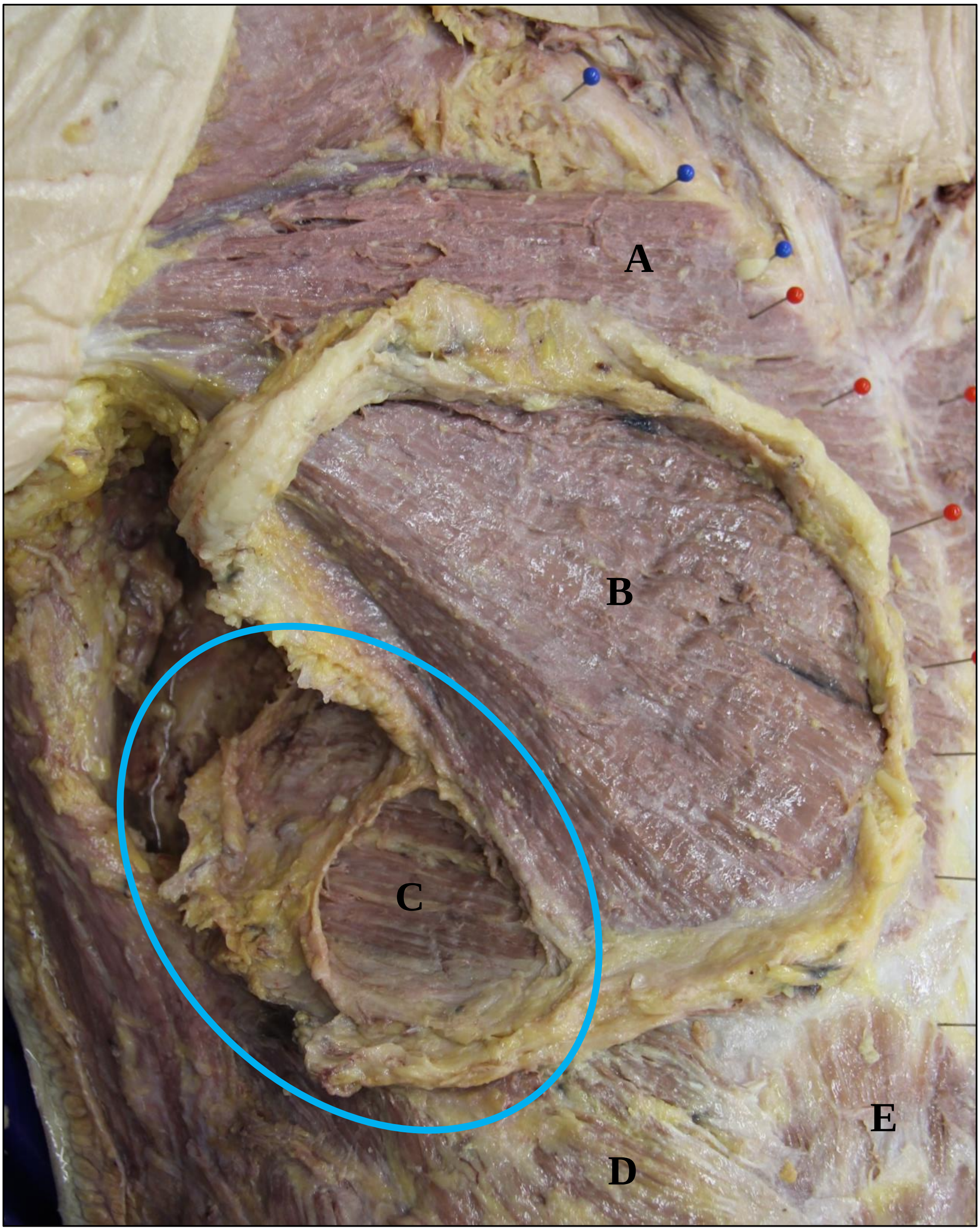

Figure G: Perimeter of the breast, with the lateral fascia confluence circled. A) Pectoralis Major muscle (clavicular head), B) Pectoralis Major muscle (sternal head), C) Serratus Anterior mucles, D) External Oblique muscle, E) Rectus Abdominus muscle.

Figure H: Footprint of the breast with the muscles inside the perimeter shaded. Pectoralis Major muscle (Red), Rectus Abdominus muscle (Yellow), Serratus Anterior muscle (Orange), External Oblique muscle (Green)

Appendix C: Conference Presentations

C.1 9th Australasian Biomechanics Conference, 2014, University of Wollongong, NSW AUS

Abstract: Anatomy of the Breast: Work in Progress

By Kathryn Gaskin[1], Dr Gregory Peoples[1], Dr Deirdre McGhee[1],

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Introduction: The anatomy of the structure of the breast appears in nearly every anatomy textbook yet it is based on data and descriptions written by Cooper in 1840[1]. Although Cooper's descriptions of the location, structure and composition of the breast were extensive, they lacked quantitative data and no detail was provided of the number of cadavers the descriptions were based on.

Since 1840, there have been only 10 anatomical studies that have verified or expanded on Cooper's work, particularly regarding the fascial and ligamentous structures of the breast. Most of these studies also lacked quantitative data. Confusion and conflict exists within both anatomy textbooks and studies regarding the terminology and location of the fascial tissue within the breasts, in particular Cooper's ligaments, the Inframammary Fold (IMF) and the attachment of the breast to the chest wall. This is despite the significance of these structures to breast surgery outcomes and the diagnosis of pathology. Due to the paucity of research on the fascial structure of the breast and the dated research that anatomical descriptions of the breast are based on, further research is required which is supported by quantitative data.

Aim: To provide extensive quantitative data on the structural components of the breast and to describe the gross structure of the female breast in terms of its structural supports and composition.

Methods: The chest plate of 10 female cadavers will be removed and both breasts dissected using two different dissection techniques. Study one will involve the left breast, which will be examined in the frontal plane, in a superficial to deep dissection. The skin around the borders of the breast will be injected to outline the perimeter of the breast to its full depth, level of the superficial pectoral fascia. Once the skin is removed, the breast will be scanned using a three-dimensional scanner (ArtecTM Eva three-dimensional Scanner, San Jose) through which breast volume and surface area will be measured. The superficial adipose and fascial layer will then be investigated macroscopically to quantify the number and volume of the adipose pockets per quadrant and the dimensions of fascial tissue bordering these pockets using anatomical grade electronic Vernier Calipers (Mitutoyo, Japan; 0.02mL accuracy). These tissues will then be removed and weighed as part of the total fibro-adipose weight (µg; Ohaus, Adventurer® Pro, USA). The anterior surface of the gland will then be exposed, outlined and scanned to quantify the surface area of the gland relative to the total breast surface area. The fibro-glandular layer will then be removed and weighed to quantify the total fibro-glandular weight. The fibro-adipose tissue posterior to the gland will then be measured in a similar manner and weighed to determine the total fibro-adipose tissue weight. The fibrous tissue attaching the breast to the chest wall and the IMF will then be dissected and described. Finally the exposed anterior surface of the chest wall will be scanned and its surface area described.

Study two will examine sagittal slices of the right breast made with a bandsaw through standardised sections of the breast. Macroscopic investigation will include the number adipose pockets, their location relative to the gland and the depth of each layer of the breast. The fascial tissue inferior and superior to the gland, the IMF and the attachment of the breast to the chest wall will also be measured and described. Microscopic investigation will be performed using a microscope (Nikon SMZ800, Australia) and a high-resolution photography (Photomicrographic system FX – III, Nikon, Australia) to quantify the surface area of each adipose pocket and the dimensions of the fascial tissue bordering these pockets.

Results and Discussion: This paper will provide state-of-the-art anatomical descriptions and quantitative data on the structural components of the breast to benefit both breast surgery and the diagnosis of breast pathology

References: [1] Cooper, S.A.P., 1840, On the Anatomy of the Breast, Volume 1. London: Longman, Orme, Green, Brown and Longmans.

C.2 Tech-Net, 2015, University of Technology Sydney, NSW AUS

Abstract: Dissection Techniques to Explore the Fibro-adipose Structure of the Female Breast

By Kathryn Gaskin[1], Dr Gregory Peoples[1], Dr Deirdre McGhee[1]

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Purpose: The anatomical terminology of the fibro-adipose structure of the female breast is conflicting and based on the outdated work of Cooper in 1840. This study aimed to investigate and quantify the structure of the breast to update anatomical descriptions for the purpose of high quality dissection.

Methods: The structure of three embalmed cadaveric breasts was investigated using dissection. The surface area (mm^2) and volume (mL^3) of each breast was measured using a three-dimensional scanner. The number of adipose pockets within the entire breast and within quadrants of the breast was manually counted. The weight of adipose tissue embedded in each pocket was weighed (g) and averaged per quadrant and per breast.

Results: The smallest breast was associated the least number of adipose pockets and the largest breast with the greatest number. Pocket size did not follow this same pattern and was not consistent amongst the different quadrants.

Conclusion: The number of adipose pockets appeared to increase with breast volume and surface area while the size of the pockets did not. The number and size of the pockets in each quadrant varied. Therefore special attention needs to be paid when dissecting the breast as each quadrant varies slightly in its fascial structure.

C.3 Australasian Institute of Anatomical Science, 2016, University of Otago, Otago NZ

Abstract: Innovative techniques to explore the structural anatomy of the breast

By Kathryn Gaskin[1], Dr Gregory Peoples[1], Dr Deirdre McGhee[1]

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Introduction: The current anatomical knowledge of the structural anatomy of the breast is based on dissections dating back to 1840. With only ten dissection studies published since, much of the current knowledge is dated and lacking any quantitative data. Anatomical texts contain limited detail of the structural anatomy of the breast and anatomical illustrations are inconsistent. The aim of this study was to explore innovative dissection techniques to investigate the fibro-adipose structure of the breast in three-dimensions.

Methods: Four innovative techniques were critiqued; (i) Viscous dye injections to outline the perimeter of the breast throughout its depth; (ii) Embedding the chest plate in expanding for and slicing it with the bandsaw to investigate the breast in the sagittal plane; (iii) Chemical dissection (NaOH) of differing temperatures and concentrations to remove adipose tissue from the fibrous structure; (iv) Three-dimensional scanning to quantify the volume, surface area of the breast and to digitally recreate the fibrous structure of the breast.

Results: (i) Viscous-dye injected along the perimeter of the breast clearly outlined the perimeter from the skin through to the ribs. (ii) Bandsaw slicing of the breast in the sagittal plane displayed the structural anatomy of the breast without disrupting any tissue. (iii) Chemical dissection only removed a percentage of adipose tissue and compromised the fibrous structure. (iv) Three-dimensional scanning was useful to

quantify breast volume and surface area but was ineffective to digitally represent the three-dimensional fibrous structure.

Conclusion: Viscous-dying, bandsaw slicing and three dimensional scanning are useful techniques to investigate and quantify the fibro-adipose structure of the female breast. Chemical dissection is not recommended to dissect adipose tissue to explore the structural anatomy of the breast as it compromised the fibrous structure.

C.4 14th Annual Conference of the Australia and New Zealand Association of Clinical Anatomists, 2016, Australian National University, ACT AUS

Abstract: An update on the anatomy of the fibro-adipose structure of the female breast

By Kathryn Gaskin[1], Dr Gregory Peoples[1], Dr Deirdre McGhee[1]

[1]Anatomy Laboratory, School of Medicine, Faculty of Science Medicine and Health, University of Wollongong, NSW, Australia

Introduction: The anatomical terminology and descriptions of the fibro-adipose structure of the female breast are inconsistent, dated and lack quantitative data. This study aimed to investigate and quantify the fibro-adipose structure of the female to update its anatomy.

Materials and Methods: The fibro-adipose structure of fifteen embalmed female breasts was dissected in the coronal plane. Outcomes measures were (i) breast and breast quadrant surface area (cm^2) and volume (m^3); (ii) fibro-adipose pockets number (N), surface area (cm^2) and mass (g). Pearson's correlations were used to investigate the association between breast volume and fibro-adipose pocket number, and breast surface area with fibro-adipose pocket number. *T* –tests compared the normalized fibro-adipose pocket number and mass of the upper and lower volume tertile ($P<0.01$).

Results: The number of pockets per breast was found to increase both with breast volume and surface area ($P<0.05$), with a strong positive correlation ($r^2=0.7372$) found between breast volume and number of pockets within the breast and between breast surface area and the total number of pockets ($r^2=0.8064$). A strong positive correlation was also found between total breast volume and total breast surface area ($r^{2=}0.9200$) ($p<0.01$)

Conclusion: This is the first anatomical study to describe the complex structure of the breast with supporting quantitative data. Although there was no significant difference in the number of pockets per breast in the largest breasts by volume (upper tertile) compared to the smallest breasts by volume (lower tertile). There was a trend however towards a higher number of pockets in the breasts of the upper tertile by volume compared to the breasts of the lower tertile breasts by volume This provides the basis for potential clinical application across a range of breast pathologies and procedures.

C.5 15th Annual Conference of the Australia and New Zealand Association of Clinical Anatomists, 2017, University of Auckland, Auckland, NZ

Abstract: Gross anatomy of the attachments and muscles involved in the 'footprint' of the female breast.

By Kathryn Gaskin[1], Dr Deirdre McGhee[1], Dr Gregory Peoples[1]

[1]Anatomy Laboratory, School of Medicine, Faculty of Science Medicine and Health, University of Wollongong, NSW, Australia

Introduction: A summary of 31 anatomy textbooks detailing the anatomy of the breast revealed limited descriptions of its attachments to the chest wall or the regional anatomy of the footprint of the breast, with no associated quantitative data. This study aimed to investigate the posterior and perimeter attachments of the breast to the chest wall and quantify the muscles involved in the 'footprint' of the female breast.

Materials and Methods: Thirteen embalmed female breasts were dissected from superficial to deep in the coronal and sagittal plane and the attachments of the breast to the chest wall were compared and described. The percentage contribution of the superficial muscles of the chest wall within the perimeter of the breast was quantified using ImageJ software (Wayne Rasband, Maryland, USA).

Results: Multiple posterior attachments of the breast to the chest wall were found that required blunt dissection only. The perimeter attachment was firmly adhered to the underlying muscle fascia and required sharp dissection along its entire length. No direct periosteal attachment was consistently found along the inferior aspect. The superficial anterior chest wall muscles within the perimeter of the breast varied with breast volume. Pectoralis Major muscle was consistently found within every perimeter and made up the largest average surface area (81%), followed by the Serratus Anterior (10%), External Oblique (5%) and Rectus Abdominus (4%) muscles.

Conclusion: This study provides gross anatomical descriptions and quantitative data on the attachments and 'footprint' of the female breast to update descriptions and illustrations in anatomical textbooks.